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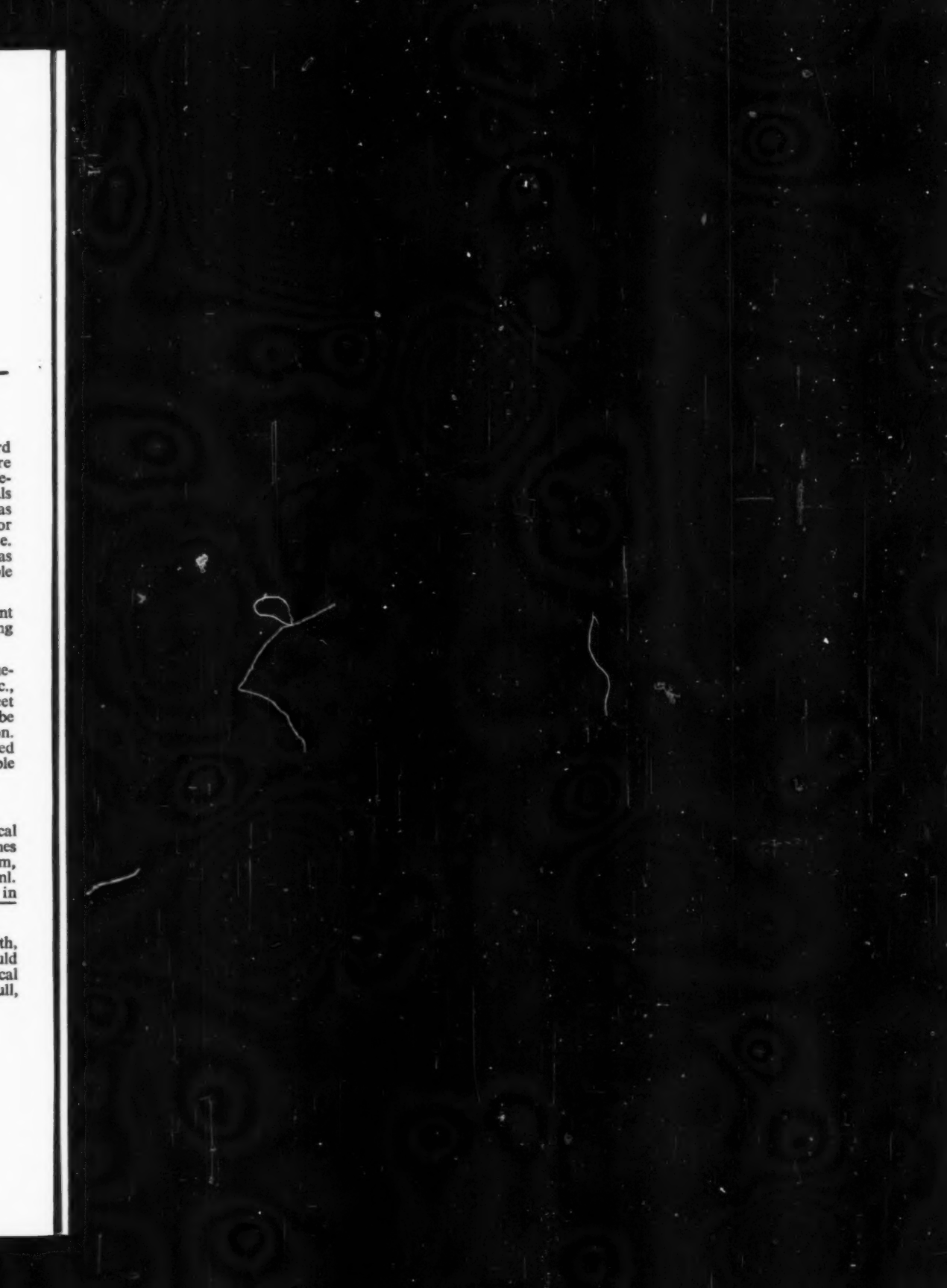

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EDITORIAL

The increasing demand for laboratory methods in the control and investigation of disease in man has produced a rapidly growing number of laboratories and specialists who are daily undertaking wider and more elaborate procedures in all four branches of pathology. Their work brings them into a special relationship with clinicians and patients, and it has become more and more difficult to extract from the wide range of journals covering these subjects the advances, experimental or established, which can be applied to the bedside.

The policy of the Association of Clinical Pathology and of the Editorial Board of the new Journal is, therefore, to provide under one cover a regular publication of such original articles, review articles, technical methods, and abstracts which will be of value to the practising clinical pathologist whose daily work must be orientated towards the elucidation of disease in man.

It is in no way intended that the Journal shall compete with the established publications, but rather that it shall augment them and in this way build a bridge between the experimental pathologist and the clinical worker.

A single number was published in July, 1945, by the Association of Clinical Pathologists, and so that these papers shall not be lost the contents are appended below:

The Examination of Wounds for Clostridia, by Nancy J. Hayward.
Heterologous Agglutinins in Diagnostic Sera, by R. W. Fairbrother.
Liver Function Tests, by N. F. Maclagan.
The Examination of Bronchial Biopsy Tissues, by S. Roodhouse Gloyne.
Interpretation of the Endometrial Biopsy, by Oliver C. Lloyd.

J. clin. Path. (1947), **1**, 2.

THE LABORATORY DIAGNOSIS OF VIRUS INFECTIONS OF MAN: A REVIEW

BY

S. P. BEDSON

London Hospital and Medical College

(RECEIVED FOR PUBLICATION, JULY 25, 1947)

The last fifteen years have seen a considerable advance in our knowledge of viruses. Admittedly it is still impossible to say whether or not viruses are living micro-organisms: there is even some doubt as to whether all those agents which we group together as viruses are of the same nature. But, interesting though these particular problems are, they have no direct bearing on the practical question of the diagnosis and control of virus disease, since for these purposes viruses can be regarded as micro-organisms. And the inability to answer these questions unequivocally does not mean that in other respects our knowledge of viruses has not made great strides. Many of the larger viruses have been stained and seen with the ordinary microscope, and the greater power of microscopic resolution obtainable by the use of ultra-violet light and a quartz optical system comprising a dark-ground condenser —an advantage which we owe largely to the work of J. E. Barnard in this country—has made possible the photography of these large viruses in an unstained and unaltered state, and has also brought some of the smaller viruses within the range of visibility. Furthermore, the introduction of the electron microscope, with its still greater powers of resolution, enables us to see even the smallest virus.

We are no longer confined to the use of animals for the cultivation of viruses. The adaptation of the technique of tissue culture to this purpose, and the use of the developing hen's or duck's egg—which we owe to the ingenuity of the American pathologist Goodpasture—have provided us with simple means for growing many of the animal viruses in the laboratory. And when we turn to the question of those serological reactions, the precipitin, agglutination, and complement fixation tests which have been of such invaluable service in the study of bacteria and bacterial disease, we find that, contrary to what was at one time thought, these methods are applicable to virus work. It is the purpose of this article to consider some of this recent work and its application to the diagnosis of diseases of man of virus aetiology.

Microscopy

Demonstration of viruses by means of the microscope.—Sixty-one years ago Buist announced that he had succeeded in demonstrating particles with the appearance of minute cocci in smears made from vaccinial lesions (see Gordon, 1937); he concluded that these were the virus. As a recent American writer has remarked: "Buist was a man far ahead of his time; his contemporaries were not merely sceptical, they were not even interested." In 1904 Borrel published a paper on the virus of fowlpox, in which he showed that, by the use of Loeffler's flagella stain, minute coccal bodies could be demonstrated in smears of virulent material, and he considered these to be the virus. Two years later Paschen of Hamburg, using a very similar technique to Borrel, rediscovered the virus of vaccinia. Although this work did not pass unnoticed it did not receive general acceptance, in fact most bacteriologists adopted a sceptical attitude which it took nearly twenty years to dispel. The reason for this scepticism was due largely to the fact that the staining methods then employed, although producing admirable preparations in the hands of the expert, gave rise to artefacts bearing a remarkably close resemblance to stained virus particles, and the tendency to dismiss all these appearances as artefacts was all too readily adopted.

Since then, improved methods of staining and the devising of technical procedures for the separation of virus from the non-specific material in the crude suspensions of virulent material have made it possible to show that the particles one can stain and see in smear preparations are, in fact, virus. In this work, fractional centrifugation has played an important part. Saline suspensions of virulent

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material are first centrifuged at a relatively low speed to throw down gross particles of tissue, and the resulting supernatant is then spun at a speed sufficient to deposit the virus. After removing the supernatant fluid, the deposit is resuspended in saline and centrifugation, first at a low speed and then at a high speed, is repeated. A deposit is finally obtained which consists very largely of particles resembling in size and staining properties those seen in the smears made from the original virulent material and thought to be virus, and the demonstration that the virulence of the original suspension has moved with the particles and that they react specifically with a known antiserum completes the proof that virus and particles are one and the same. There is, therefore, no doubt that the larger viruses can be seen with the microscope. They look like minute micro-organisms, and this conception of their nature finds support in the dark-ground photomicrographs that Barnard has produced of unfixed and unstained viruses.

Turning now to the practical application of this work to the diagnosis of virus disease, it is obvious that direct microscopy can be of use only in the case of the large viruses. Even here the limitations of the method are greater than in bacterial infections. The largest viruses are so much smaller than bacteria that they can be detected only if they are present in considerable numbers, and the occasions when their identity can be assumed because they have been seen in smear preparation are very few. However, the method is not without value and it finds its use particularly in the diagnosis of infections due to that group of large viruses which are distinguished from the others by staining by Castaneda's or Macchiavello's methods. For that matter, this group possesses other characteristics that mark it off from the rest: the viruses are larger, and when they multiply they present a regular sequence of morphological change, recalling those changes in size and shape which a bacterium often undergoes when multiplying. This group contains amongst others the agents of trachoma, inclusion conjunctivitis or inclusion blenorrhoea, lymphogranuloma venereum, and psittacosis. The use of microscopy in confirming the diagnosis of trachoma, particularly in its early stages, is well known. Halberstaedter and von Prowazek (1907) were first to demonstrate the characteristic cytoplasmic inclusions in the conjunctival epithelium in this condition, and the more recent work of Thygeson and Proctor (1935) has shown that the particles which occur both free and inside the cells and which are readily stained by Castaneda or Giemsa are, in fact, the virus.

Inclusion conjunctivitis.—Infection with the closely related virus of inclusion conjunctivitis is of greater interest to us, since it is of commoner occurrence than trachoma in this country, This virus is morphologically indistinguishable from that of trachoma; the exact relationship of the two, though obviously close, is yet to be determined. Like the trachoma virus, it is a parasite of man and is found producing infection of the conjunctiva and the mucous membrane of the cervix and the male urethra. Cells infected with this virus present inclusions which in situation, structure, and staining properties bear an extremely close resemblance to those of trachoma. They consist of masses of virus particles held together by inclusion material which is basophilic and scanty in amount. These inclusions differ, therefore, from what one might call the "typical" virus inclusion, in which the inclusion material is acidophil and present in such amount as completely to mask the virus, and it is this scantiness of inclusion material in the inclusions produced by the group of Castaneda-positive viruses which has made it so much easier to study and recognize their true structure. Like the other members of this group, the virus of inclusion conjunctivitis shows the sequence of developmental forms to which reference has already been made. The large forms, which are almost the size of staphylococci, have been called by Lindner (1910) "initial bodies," since in his opinion, they represent the initial stage in the development of the virus, a view which is fully supported by recent work. As the virus multiplies—and all appearances suggest that it does so by simple division—smaller and smaller forms are produced until, when the colony is fully grown and the cell is ready to break down and discharge its virus content, all the virus is in the form of elementary bodies.

Inclusion conjunctivitis occurs either as a neonatal infection, when it is acquired in precisely the same way as gonococcal ophthalmia, or in older children or adults, when it is often called swimming-bath conjunctivitis. Both forms are associated with inflammation of the conjunctiva and some discharge; scattered follicles may be present. Diagnosis is made by the microscopy of conjunctival scrapings suitably stained, and some idea of the incidence of the neonatal form can be gathered from a recent paper by Sorsby and others (1944), who found twenty-seven cases in 269 of all forms of conjunctivitis in infants.

Cervicitis and urethritis.—Infection of the mucosa of the female genital tract is usually silent, but it can be revealed by the examination of stained smears of cervical scrapings. In the male, infection of the

urethra takes a subacute form which may become chronic and persist for months; this infection of the genital tract is transmitted by sexual intercourse and, although it undoubtedly occurs in this country, it appears to be uncommon.

Psittacosis.—The virus of psittacosis is another that can be recognized microscopically; and, although it is not suggested that direct microscopy is of value as a diagnostic procedure in human infections with this virus, it is undoubtedly of use in the case of infection of birds. It is often possible to find the virus in smears made from the spleen, which is commonly enlarged, or with material from the air sacs, where infection often resides, and so to make an early diagnosis.

Lymphogranuloma venereum.—Lymphogranuloma venereum virus, another member of the Castaneda-positive group, can also be detected in suitably stained smears of the pus from the inguinal buboes which occur in the male and when the primary lesion is penile, or when it is situated in the anterior part of the genital tract of the female. Though possibly worth making, this investigation has not the same value in the diagnosis of lymphogranuloma venereum as isolation of the virus, the Frei test, or the complement fixation reaction.

Smallpox.—As a further example of the diagnostic value of the microscopical demonstration of virus, mention might be made of its use in smallpox. This virus is of a size readily visible under the microscope, and a number of staining methods are available for its demonstration, some of which, such as the alkaline methyl-violet method of Gutstein (1937), are quite simple. The virus is present in quantity in the skin lesions and, as van Rooyen and Illingworth (1944) have shown, microscopic examination of suitably stained smears made from scrapings obtained from the base of papules or vesicles can be of considerable diagnostic value. In the cutaneous lesions due to herpes febrilis, zoster, or varicella, the virus particles, though present and staining by the methods applicable to variola, are neither so numerous nor so large as variola virus. Van Rooyen and Illingworth used Paschen's staining method. Commenting on this diagnostic procedure, Downie (1946) states a preference for Gutstein's stain and points out that smears made from pustular lesions or crusts are unreliable, that a negative result does not exclude smallpox, and that the test would not differentiate between the lesions of smallpox and vaccinia.

Demonstration of specific histological changes.—Quite apart from the possibility of demonstrating the virus itself, there are, in many virus diseases, demonstrable histological changes in the infected cells which are sufficiently distinctive to be of diagnostic value. These are the inclusion bodies found in the cytoplasm or the nucleus of the affected cells, bodies of varying size, usually homogeneous in appearance and acidophil in staining reaction, which may come to occupy the major part of the nucleus or a considerable part of the cell's cytoplasm. Doubt still exists as to the exact nature of the nuclear inclusions, but it has been shown that many cytoplasmic inclusions are virus colonies, masses of virus embedded in a matrix of homogeneous material. It is this latter material which gives to many of these inclusions their appearance of homogeneity and affinity for acid dyes. In the case of the cytoplasmic inclusions produced by the Castaneda-positive viruses, inclusion material is in insufficient quantity to mask the virus particles, and these can usually be readily seen either in Castaneda- or Giemsa-stained preparations. Even where the inclusions appear homogeneous when fixed and stained, it is still possible, as Barnard has shown in the case of ectromelia, to demonstrate the true nature of the inclusion by using dark-ground illumination. The difference in refractility of virus and inclusion material shows up the former as bright dots.

At one time it was thought that only infection with a virus could produce inclusion bodies, but it is now known that very similar appearances can be produced experimentally by other means. This applies particularly to nuclear inclusions. None the less, the finding of inclusion bodies can be taken as presumptive evidence of a virus infection, and in some instances their demonstration has diagnostic value. The finding of Negri bodies, the acidophil cytoplasmic inclusions produced by rabies virus in nerve cells, confirms the diagnosis of suspected rabies in the dog. And mention has already been made of the demonstration of the Halberstaedter-Prowazek bodies in trachoma and the similar inclusions in inclusion conjunctivitis, cervicitis, and urethritis as diagnostic procedures. Paul's test for smallpox, which depended on the production of lesions in the rabbit's cornea characterized by cytoplasmic inclusions, has been largely superseded by the demonstration of the infective agent in the skin lesions either by direct microscopy or by the complement fixation test.

Isolation of the Virus

If the application of microscopy to the diagnosis of virus infections has been dealt with at some length this has been done deliberately in order to

emphasize that, contrary to common belief, many viruses are sufficiently large to be seen and recognized by means of the microscope. The limitations of this procedure have not, however, been lost to sight, and, in virus infections as in any other infective process, the aim of the laboratory should be the isolation and identification of the infective agent. Unfortunately this is less easy to achieve than in bacterial disease. Not only is the isolation and identification of a virus a lengthy and often difficult procedure, involving the infection of animals or embryonated eggs, the study of the histological lesions produced by this infection, and the application of serological tests usually in the form of the neutralization reaction, but there are quite a number of viruses infecting man for which a suitable experimental animal has not been found. The viruses of zoster, varicella, and infective hepatitis exemplify this: to them, man alone would seem to be susceptible. Or it may be that the only susceptible species of animal is so expensive as to preclude its routine use; it is this factor, probably more than any other, which has delayed our understanding of poliomyelitis and its epidemiology. Even where a virus can be isolated by the use of the incubated egg or an inexpensive animal, the time taken to establish and identify it is too long to make the procedure of immediate diagnostic importance. That does not mean that every attempt should not be made to isolate the agent of a suspected virus infection, because this should, of course, always be done where the diagnosis is in doubt. The following examples illustrate some of the commoner uses of this form of investigation.

Egg inoculation.—It is at times important to know whether an outbreak of clinical influenza is due to one of the known influenza viruses and, if so, which one. This might be necessary, for instance, at the commencement of an epidemic when prophylactic immunization is contemplated; and, though at one time the ferret would have been chosen as the most suitable animal for the purpose, probably the incubated hen's egg would now be the choice. Hirst (1942 and 1945) has found the injection of naso-pharyngeal washings into the amniotic sac of thirteen-day eggs slightly superior to ferret inoculation for the isolation of influenza A virus, an observation confirmed by Burnet and others (1942). And Beveridge and others (1944) employed the same method successfully for the isolation of influenza B virus. The combination of penicillin and sulphadiazine with the crude washings does away with the necessity for filtration. The embryonic membranes of the developing hen's or duck's egg—the amniotic, allantoic, and yolk sacs and the chorio-allantois—provide admirable means for the cultivation of a number of viruses to which man is susceptible; in addition to influenza A and B, the viruses of herpes simplex, psittacosis, lymphogranuloma venereum, mumps, measles, lymphocytic meningitis, and smallpox can all be grown on one or other of these membranes. Incidentally, North and others (1944) utilized inoculation of the chorio-allantois with suspensions of skin crusts in the diagnosis of smallpox, a procedure the value of which has been fully confirmed by Downie (1946) and Downie and Dumbell (1947).

Value of animal inoculation.—Invaluable though the egg technique has proved in virus work, it has by no means superseded the use of experimental animals, for there are occasions when only the latter are suitable to the work in hand. This is true, for instance, of poliomyelitis, where inoculation of the monkey is our only means of demonstrating the presence of the virus. Even in those cases where the virus sought will infect both eggs and animals it is advantageous to use both; it increases the chance of success and it may well be that the disease produced in the animal is more distinctive than the changes resulting from infection of the egg. The latter may, in fact, be so inconspicuous as to make their detection difficult and uncertain, as in infection of the chorio-allantois with the virus of lymphocytic chorio-meningitis. Or, as recent work on rinderpest has shown (Shope and others, 1946), a virus may multiply within the developing egg without producing any detectable naked-eye or histological changes or even death of the embryo.

Meningitis.—So where both egg and animal are available for our purpose we use both. This is true, for instance, in the investigation of acute aseptic meningitis, a syndrome due to a number of viruses, some of which have been identified. Of these, the virus of lymphocytic choriomeningitis of Armstrong and Lillie is responsible for about one-third of cases. At any rate, that would appear to be so in North America, though one has the impression that it is a less frequent cause of benign meningitis in this country. Other viruses which have been found producing this form of meningitis are the pseudo lymphocytic choriomeningitis virus of MacCallum, Findlay, and Scott (1939) and the virus of lymphogranuloma venereum. Infection with the last-named virus may rarely present as a meningitis (Sabin and Aring, 1942), and the virus of MacCallum and Findlay has been isolated on only *two* occasions; more than half the cases of this condition are due to a virus or viruses yet to be identified. The three

known causal agents are to be found in the cerebrospinal fluid, and all these are pathogenic for the mouse and guinea-pig. Wherever the diagnosis of acute aseptic meningitis is in question, cerebrospinal fluid collected as early in the disease as possible should be injected intracerebrally in mice and subcutaneously in the thigh of guinea-pigs. Should any of the three viruses be present, encephalitis may develop in one or more of the mice, and the virus so isolated can be established and studied. The virus of Armstrong and Lillie (1934) will often infect the guinea-pig, producing a generalized infection with pyrexia, wasting, and salivation, the animal dying usually in from nine to sixteen days. Should the case of meningitis prove to be tuberculous, and in the early stages of this condition the differential diagnosis from benign lymphocytic meningitis of virus origin may well arise, the mice will remain well whereas the guinea-pigs will develop tuberculosis.

Lymphogranuloma venereum.—In addition to producing an encephalitis when inoculated intracerebrally in mice, lymphogranuloma virus also produces a local lesion when injected sub-cutaneously in the guinea-pig's thigh, and an enlargement of the inguinal lymph nodes occurs; the lesions can be examined histologically; and, by further passage in animals and eggs, the virus can be established and identified. The use of monkeys in such an investigation has not been mentioned because they are expensive and not essential, though, if available, one or possibly two might be inoculated intracerebrally. And it has been assumed that eggs, if available, will be used in parallel with the animals.

Psittacosis group.—Another example of the use of animal inoculation, in addition to eggs, in the diagnosis of virus infection is provided by diseases caused by viruses of the psittacosis group. Human infection with psittacosis virus is often associated with some degree of pneumonia, and either sputum or lung puncture material can be used for animal inoculation, though in psittacosis pneumonia sputum is often scanty. The mouse is the animal usually employed; and, if sputum is the material chosen, the presence of bacteria, particularly the pneumococcus, may introduce a complication. Though the bacteria can be got rid of by filtration, this also reduces the amount of virus, and a better procedure is to protect the mouse against the bacteria by means of sulphonamides, to which psittacosis virus is insensitive, or relatively so. This procedure has the additional advantage, if the mice have been inoculated by the nasal route, of preventing a lighting up of latent infection of the mice with the virus of Nigg (Nigg and Eaton, 1944), present in many mouse stocks. This virus belongs to the Castaneda-positive group and is fortunately susceptible to sulphonamides. And, in connexion with the use of mice for the isolation of psittacosis virus, it is important that both the intranasal and intraperitoneal routes of inoculation should be employed, because certain strains of psittacosis virus produce only an inapparent infection when introduced into the peritoneal cavity. This is true of pigeon strains, and here it might be pointed out that the host range of this virus is much more extensive than was at one time thought. In addition to birds of the parrot tribe, certain species of finch, fulmar petrels, pigeons, the domestic fowl, and ducks have been found to suffer from natural infection with psittacosis virus and to be responsible for human infections.

Serological Investigation

It is still far from being widely appreciated that those serological tests which are in daily use in the identification of bacteria and the diagnosis of bacterial disease are equally applicable to viruses. Virus workers themselves have been to some extent responsible for the tardy recognition of this fact. There is no need here to consider the reasons for this; it is sufficient to note that it is now well established that the precipitin, agglutination, and complement fixation tests are all available for the investigation of viruses and the diseases they cause. And, because it is more difficult to obtain a suspension rich in virus than is the case with a bacterium, the complement fixation test is the serological reaction which has been found most suitable for virus work, since, as Merril has shown (1936), this test has a lower antigen threshold than the precipitin or agglutination reactions. A serological test could be used in the diagnosis of a virus infection either for the purpose of demonstrating the presence of virus in the infective material or for the detection of antibodies formed in response to the infection.

Demonstrating virus in infective material.—As an example of the former we have the use of the complement fixation test in the diagnosis of smallpox. Craigie and Wishart (1936) have shown that this is a reliable test for the presence of variola antigen in the vesicle fluid or in an extract made from the crusts of older skin lesions; the serum employed is one made against vaccinia virus in the rabbit. Tulloch (cited by Downie, 1946) has had wide experience of this test in the outbreaks of smallpox which occurred in Scotland in the war

of 1939–45; he considers the test to have a high diagnostic value. This opinion is shared by Downie (1946), who found that vesicle fluid or crusts from twenty-two cases suspected of being smallpox on clinical or epidemiological grounds gave a positive result; his material came from twelve different outbreaks. Similar material from other conditions, such as varicella, zoster, or septic skin rashes, gave a negative reaction. He concludes that this is the most valuable single laboratory test available for the diagnosis of smallpox, an opinion which seems fully justified by the evidence.

This is one of the few examples, however, of this particular use of serological reactions in the diagnosis of human virus infections. It has, at times, been applied, in conjunction with animal inoculation, for the differentiation of zoster from herpes simplex, using a known positive convalescent zoster serum and the fluid from the vesicular eruption (Bedson, unpublished observations).

Detection of antibodies.—But, generally speaking, the concentration of antigen in infective material from virus infections is too low even for its detection by the complement fixation reaction, and it is for this reason that the indirect use of such a test (that is, for the demonstration of specific antibody in the patient's serum) is of so much wider application as a diagnostic procedure. It would be impossible here to give a comprehensive survey of this use of the complement fixation test; a few illustrative samples will have to suffice.

Lymphocytic choriomeningitis.—In the course of infection with the virus of lymphocytic choriomeningitis, complement fixing antibodies are developed (Smadel and others, 1939) for the soluble antigen of this virus. Although these antibodies have probably nothing to do with immunity to this virus, they are specific and their presence diagnostic (Smadel and others, 1939; Lépine and Sautter, 1938). The soluble antigen is present in considerable amount in the tissues of the experimentally infected animals, and a suspension of the spleens of guinea-pigs succumbing to lymphocytic choriomeningitis provides a satisfactory antigen which remains stable when stored in the refrigerator for months or even a year or more. It is important to note that a freshly made spleen suspension may give false reactions, it should be allowed to age for two to three weeks before use. Although the American and French work already referred to leaves no doubt as to the specificity and diagnostic value of this test, limited experience of its use in this country has been disappointing (Bedson, unpublished findings). During the war a number of sera from cases of benign aseptic meningitis have been examined without obtaining a single positive result, and, although in some of these cases this was probably due to their not being caused by the virus of Lillie and Armstrong, there were two from which this virus was isolated by mouse inoculation.

Influenza.—The complement fixation test can also be applied to the diagnosis of influenza (Smith, 1936; Hoyle and Fairbrother, 1937; Fairbrother and Hoyle, 1937; Francis and Magill, 1937; Tulloch, 1939), but since antibodies to influenza virus are to be found in detectable though low titre in the blood of most adults and older children, diagnosis depends on demonstrating a significant increase in these antibodies, and that makes the findings mainly of retrospective interest. This being so the neutralization test, which takes so much longer to do, might equally well be employed, and in the past it is the neutralization test which most workers have used. It may be, of course, that the Hirst phenomenon of agglutination of the red cells of the domestic fowl by influenza virus (1941), which is specifically inhibited by influenzal antibody and can be used as a method of antibody titration, will eventually largely replace both complement fixation and neutralization tests.

Virus agglutination of red cells.—The subject of virus agglutination of red cells, which, since Hirst's original work, has been the object of much study, principally by Burnet and his colleagues in Australia, leads to mention of a recent observation from Burnet's laboratory. This is not the place for any detailed consideration of all the interesting work done in this field; suffice it to say that the phenomenon of red-cell agglutination is not confined to the influenza virus and chick cells; other viruses will do this, and the red corpuscles of certain species of mammal, including man, are agglutinated as well as chicken red cells. The absorbed virus responsible for the agglutination is liberated after a few hours; this fact, incidentally, is utilized in the concentration of the influenza viruses in the preparation of influenza vaccine.

The recent observation of Burnet and Anderson (1946) to which it is desired to draw attention is that human red cells which have been agglutinated by the virus of Newcastle disease of fowls and from which the virus has been eluted have become susceptible to agglutination by the majority of sera from recent cases of glandular fever. This work is still in its initial stages. The evidence produced by Burnet and his colleague suggests that the change in

the cells is due to the absorption of antigenic material, other than virus, produced by Newcastle disease virus when growing in chick embryo cells. Burnet (1946) has also shown that human red cells treated with egg-grown mumps virus provide a sensitive test for mumps antibody in human sera. Although much remains to be done, this work opens up such exceedingly interesting possibilities that it seemed well worth mentioning.

Psittacosis-lymphogranuloma venereum group.—It is perhaps in the diagnosis of infections with the viruses of the psittacosis-lymphogranuloma venereum group that the complement fixation test has been most widely applied. This test has been used in the diagnosis of psittacosis in man (Bedson, 1935 and 1937) for eleven years or more, and the work of Meyer and his colleagues and others in America has confirmed its value. By means of this test support was given to the findings of Haagen and Mauer (1939) that cases of an atypical pneumonia occurring in the Faroe Islands were due to psittacosis or some very closely related virus, and it was used to show that a similar clinical condition in Iceland had a like aetiology (Bedson, 1940). Infection in these cases originated in fulmar petrels. Some involvement of the lung is of frequent occurrence in human infections with psittacosis virus, and it has long been recognized that the resulting pneumonia was unlike bacterial pneumonia in its clinical signs and histological picture. It was not, therefore, very surprising when Eaton and others (1941) showed that some cases of the condition known as primary atypical pneumonia, which in the recent war assumed almost epidemic proportions in this country and elsewhere, were due to psittacosis virus. This observation was based on isolation of the virus from sputum or lung material and the demonstration of a positive complement fixation test with the patients' sera. Subsequent work has shown that an appreciable proportion of cases presenting this syndrome, which Reimann (1946) quite rightly prefers to name "virus pneumonia," are due to viruses belonging to the psittacosis group. Smadel (1943), for instance, found that some 15 per cent of forty-five cases of so-called primary atypical pneumonia were probably due to a psittacosis virus, and, in a serological survey made of this condition in England during the war, nine cases out of 120 had a very high or rising titre of antibody for psittacosis virus (Bedson; unpublished findings).

The more extensive use of the psittacosis complement fixation test resulting from these investigations has shown that the mere presence of such an antibody in the serum of an individual does not necessarily mean active infection with a virus of this group. As in the use of any serological test for antibody as a diagnostic procedure, the demonstration of a rising titre of antibody is necessary to postulate active infection. Smadel (1943) rightly insists that only a fourfold rise in psittacosis antibody should be accepted as evidence of active infection, and with this the writer would fully concur. The recent work in America showing that the viruses belonging to the Castaneda-positive group are closely similar in antigenic structure has introduced a further complication. Rake and others (1941) found that sera from patients infected with the viruses of lymphogranuloma venereum and the psittacosis group all cross-reacted when tested by the complement fixation test with these viruses. They further showed that cases of atypical pneumonia due to a psittacosis virus might give a positive Frei test. And the demonstration by Rake and others a year later (1942) that the sera from cases of trachoma and inclusion conjunctivitis may fix complement with the antigens of lymphogranuloma virus suggests that these two viruses also share antigens with the psittacosis-lymphogranuloma group. There is further evidence obtained from cross-immunity experiments in animals confirming the close relationship between the viruses of the psittacosis group—parrot psittacosis, pigeon psittacosis or ornithosis, and the virus isolated by Eaton and others from atypical pneumonia—and the virus of lymphogranuloma venereum. How close this relationship will prove to be remains to be seen, but the immediate practical bearing of this work is that the serological tests which have been accepted in the past as specific for psittacosis and lymphogranuloma venereum can no longer be so regarded. And the Frei test, as one might expect, would appear to be no more specific than the serological tests. Though this does not mean that these tests are without value, it does emphasize the importance, which of course always existed, of invariably interpreting them in conjunction with the clinical findings.

General Remarks

This account of the part which the laboratory can play in the diagnosis of virus infections is far from complete. Only the more important examples of this have been mentioned, and they have been given shorn of much detail and supporting evidence. It is hoped, however, that this paper will have shown that the laboratory can be of help, not just occasionally by making some special investigation, but in an everyday routine way.

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MENINGOCOCCAL MENINGITIS WITH PARTICULAR REFERENCE TO EPIDEMIOLOGY AND PATHOGENESIS

BY

R. W. FAIRBROTHER

Department of Clinical Pathology, Manchester Royal Infirmary

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In this country cerebrospinal meningitis (fever) is an endemic disease found mainly in children and adolescents; local outbreaks occur, but they are rare and usually involve few individuals. The disease has, however, a marked predilection for war-time conditions; relatively extensive outbreaks occurred during the 1914–18 war, and again to an even greater extent during the 1939–45 war. These outbreaks involved individuals of all ages, including Service personnel, but it is interesting to note that, even though there was a great increase in the total number of cases notified, these tended to occur singly and an epidemic, in the generally accepted sense, did not develop.

Although such general factors as the enhanced virulence of the meningococcus and the resistance of the individual are well recognized as playing an important role in the epidemiology of the disease, a precise explanation of the peculiar nature of the outbreaks is still lacking. A popular theory is that outbreaks of cerebrospinal meningitis result from a widespread meningococcal infection of the nasopharynx in the general population (Ministry of Health Memorandum, 1940). Many workers accept the hypothesis developed by Dopter (1921) that meningococcal infection occurs in three progressive stages: (1) catarrhal, (2) septicaemic, and (3) meningitic; in the majority of cases the infection is arrested at the catarrhal stage and the occurrence of meningitis is a rare phenomenon (Lundie and others, 1915; Herrick 1918; Murray, 1929; Brinton, 1941). The evidence offered to support this hypothesis is, however, inconclusive and unconvincing. Furthermore this theory does not provide a solution of many problems presented in the various outbreaks, and it seemed desirable that the question should be re-examined in the light of information gained during the 1940–42 outbreak.

During the past seven years an opportunity has been available of studying the disease under Service and civilian conditions during both so-called epidemic and interepidemic periods and it was possible to direct attention particularly to the epidemiology and pathogenesis of the disease. The results are discussed below; they do not support the above hypothesis and an alternative is offered.

Technique

Cerebrospinal fluid.—Samples of cerebrospinal fluid were centrifuged as soon after collection as possible for 5 or 10 minutes; the supernatant fluid was decanted and the deposit seeded heavily on to Loeffler serum slopes and blood-agar plates, films were then made and the remainder of the deposit added to 10 ml. of glucose-broth, usually containing a small amount of para-aminobenzoic acid. Incubation was carried out at 37° C.; the blood-agar plates were usually placed in an atmosphere containing approximately 10 per cent carbon dioxide.

Nasopharyngeal swabs.—Material was collected from the nasopharynx by means of the West swab, which was pressed and then stroked firmly against the mucous membrane. Cultures were prepared on blood-agar plates; after overnight incubation under ordinary atmospheric conditions, suspicious colonies were sub-cultured on to Loeffler slopes for further investigation; the plates were invariably incubated a further twenty-four hours and re-examined.

Blood cultures.—Ten ml. of blood were collected by venepuncture; 2 ml. were added to a small amount of trypsin broth before mixing with 10 ml. agar, 2 ml. to Robertson's meat medium, and the remainder was placed in 25 or 50 ml. of glucose broth. Incubation at 37° C. was continued for seven to fourteen days before a negative report was given, subcultures on Loeffler serum slopes being made after forty-eight hours and later as indicated.

The identification of the meningococcus was carried out by fermentation tests with sucrose, maltose, and

glucose in Hiss's serum water and finally by agglutination tests using Standard Group sera. The suspensions for the agglutination tests were made by emulsifying the growth from a Loeffler slope in normal saline and adjusting to a light opacity; the tube method with incubation at 55° C. was used. Standard Group sera, from Army and M.R.C. sources, were used throughout.

Preliminary tests were frequently carried out by the slide agglutination technique; useful information was obtained when the strains readily agglutinated, but this method proved unsatisfactory with relatively inagglutinable organisms.

Scope of the Investigation

Material was collected from various sources during this investigation, particularly from cases, close contacts of cases, and members of the general community (Table I).

TABLE I

SOURCE OF MATERIAL

Type of case		Number
Meningitis	service	105
	civilian	47
Purulent conjunctivitis		2
Chronic septicaemia		3
? Carrier		
	close contacts	144
	general community	530

Cerebrospinal fluid only was examined from the civilian cases of meningitis, but the Service cases were investigated in greater detail. Cerebrospinal fluid was examined from all, while blood cultures and nasopharyngeal swabs were collected from a limited number. The search for carriers was carried out by the collection of nasopharyngeal swabs. Close contacts (Service personnel) were considered to be individuals occupying beds adjoining the case or all occupants of small rooms or tents in which the case was sleeping (War Office Memorandum, 1940). While such a definition did not embrace all close contacts, it did ensure that all members of this group had been exposed to the nasopharyngeal discharge of the patient a short time before the collection of the swab.

Clinical Course and Therapy

The various clinical manifestations of meningococcal infections have been described in detail by Rolleston (1919), Priest (1941), and Brinton (1941). The cases seen during this investigation followed the general pattern. The cases of meningitis during the 1940–42 outbreak were mainly of a severe type, the initial symptoms being headache, neck rigidity, vomiting, and malaise. Some examples of the fulminating meningeal type were seen; these within a few hours of the onset of the disease exhibited mental confusion and delirium. During the later stages of the outbreak the mild abortive form of the disease was occasionally encountered. No examples of adrenal involvement (Waterhouse-Friderichsen syndrome) or of encephalitis (Banks and McCartney, 1942) were seen.

Intensive chemotherapy with a sulphonamide, usually sulphapyridine, was begun immediately the diagnosis was established on the lines recommended by Banks (1939). Serum was not used. The usual practice was to examine the cerebrospinal fluid before chemotherapy was instituted; the fluid was invariably turbid and meningococci generally demonstrable.

In the fulminating types of disease the soluble form of the sulphonamide was administered by the intravenous route.

The results were excellent; in the group of 105 Service cases there was only one death. This is very much lower than the general mortality rate for the disease, but it must be appreciated that the cases were adults of military age and that therapy was usually instituted early in the disease; it is well known that the mortality rate is highest at the extremes of life (Jubb, 1943; Beeson and Westerman, 1943).

Several cases of chronic meningococcal septicaemia were suspected on clinical grounds, but in only three instances was it possible to confirm the diagnosis by blood culture. When this was accomplished sulphonamide therapy was instituted with the anticipated dramatic effect.

Bacteriology

Meningococci isolated during this investigation generally behaved in the characteristic manner which was described in detail by Murray (1929). A few interesting features were, however, observed, and these merit special consideration.

The standard solid media used throughout the investigation were 6 per cent blood-agar plates and Loeffler serum slopes. Both media gave a satisfactory growth but it was observed that, under ordinary atmospheric conditions, strains isolated in pure culture directly from the cerebrospinal fluid grew readily on the Loeffler serum slopes in screw-cap bottles, while growth on blood-agar plates was not infrequently poor; incubation of the blood agar plate in 10 per cent carbon dioxide, however, gave a luxurious growth. It therefore follows

that, if blood-agar is used for the isolation of the meningococcus from cerebrospinal fluid, incubation should be carried out in an atmosphere containing carbon dioxide.

Biochemical tests were limited to the fermentation of glucose, maltose, and sucrose in serum-water. The great majority of strains gave consistent results, glucose and maltose being fermented but not sucrose; irregularities were occasionally found.

The agglutination tests followed the generally accepted rule that strains isolated during an "epidemic" period agglutinate readily with standard sera, but during "non-epidemic" periods inagglutinable strains tend to appear. During the main 1940–42 outbreak, cerebrospinal fluid strains agglutinated readily with the Standard Group sera; many gave clear-cut agglutination only with Group I serum, but some did not exhibit any marked antigenic specificity and agglutinated also to varying degrees with Group II serum. Many indications of the complex antigenic structure of the meningococcus were obtained during this investigation, but it was not possible to examine this problem. A simple classification by means of Standard Group sera only was attempted.

When the large outbreak had subsided and infrequent sporadic cases only were reported, a different serological picture was seen. Thus during the 1946–7 winter several severe cases, mainly in infants, were investigated; meningococci were isolated from the cerebrospinal fluid but these tended to agglutinate poorly, usually giving a slight reaction only with the Group II serum; one strain proved to be inagglutinable with the sera available (Table II).

Nasopharyngeal swabs gave similar results; strains isolated during the main outbreak were more easily grouped than those obtained during the non-epidemic period. Throughout the investigation Group II strains were predominant.

Epidemiology

The epidemiology of cerebrospinal meningitis presents several unusual features for which no adequate explanation has been offered. The marked predilection of the disease for war-time conditions has long been recognized, and therefore the sudden widespread outbreak of the disease at the beginning of 1940 was not unexpected. It is interesting to note that the incidence of the disease in this country during 1940 reached the unprecedented total of 12,771 cases; this figure is actually greater than the total figures for the 1914–18 period. There was not, however, an epidemic in the generally accepted sense of the term; cases were widely distributed, occurred sporadically, and involved individuals of all ages. Fortunately the sulphonamides proved excellent therapeutic agents and some of the serious problems of 1914–18, such as the high mortality rate and long periods of hospitalization, did not arise.

During the 1914–18 outbreak special research teams carried out extensive investigations of the disease and the following important fundamental points were established: many normal individuals harbour the meningococcus in their nasopharynx; cases of cerebrospinal meningitis develop almost entirely from contact with a carrier (instances of infection from other cases of the disease occur but they are relatively rare); the disease is transmitted by droplet infection, and consequently overcrowding and poor ventilation play prominent roles in the spread of infection.

The precise significance of the carrier is uncertain. Glover (1920) claimed that a carrier rate of over 20 per cent in a community was not only an index of overcrowding but also a warning of an impending outbreak of meningitis, and he stressed the importance of the search for carriers as a prophylactic measure. Subsequent observations have, however, failed to confirm this rather extreme view. Dudley and Brennan (1934) in a naval garrison found a carrier rate of 13 per cent associated with 11 cases of meningitis, while at a later period the carrier rate was 55 per cent but no case of meningitis developed. Other workers have also found carrier rates over 20 per cent without any increased incidence of meningitis (Straker et al., 1939). It is, therefore, obvious that the development of meningitis in an individual is dependent on factors other than the presence of carriers in the community, and there is little doubt that these are closely concerned with (1) the virulence of the meningococcus, and (2) the resistance of the host.

Virulence of meningococcus.—The absence of suitable animals has prevented a detailed study of this problem and it has been necessary to depend mainly on indirect serological tests for information. During the 1914–18 outbreak four serological types were identified (Gordon and Murray, 1915); these types were not sharply demarcated and considerable antigenic overlapping occurred. In view of this other workers (Griffith, 1918; Scott, 1918) considered that the most practical serological classification was into two main groups—Group I embracing types I and III, and Group II comprising types II and IV. It was generally recognized that inagglutinable strains were particularly prominent in the nasopharynx during interepidemic periods.

The use of Group sera is now the accepted method of subdividing meningococci, but knowledge of the antigenic structure of the meningococcus is still incomplete and irregularities are not uncommon.

In the 1914–18 outbreak, the majority of cases were caused by types I and II, but since that time there has been a close relationship between Group I strains and major outbreaks. It is therefore not surprising to find that, while Group II strains have predominated in carriers, some 90 per cent of strains isolated in this country from cerebrospinal fluid during the 1940–42 outbreak belonged to Group I (Harries, 1942; Ministry of Health Report, 1946).

During this investigation there was striking serological difference in the strains isolated during the 1940–42 outbreak and those isolated from the infrequent cases seen during the 1946–7 winter (Table II).

TABLE II

SEROLOGY OF MENINGOCOCCI ISOLATED FROM CEREBROSPINAL FLUID

	1940–2	1946–7
Group I	86	1
Group II	3	9
Inagglutinable	1	1
Total	90	11

The close association of Group I strains with major outbreaks or "epidemics" suggests strongly that such strains are more virulent than those of Group II. It is, however, the general experience that there is little or no difference in the severity of the disease produced by these strains. It must be appreciated that normal cerebrospinal fluid is an excellent culture medium for most bacteria and, provided a sufficient number is introduced, multiplication will occur irrespective of the intrinsic pathogenicity of the organism.

An important difference in the two serological groups of meningococci is therefore the greater facility with which Group I strains are able to pass from the nasopharynx to the cerebrospinal fluid. The factors concerned with the virulence of the meningococcus are obscure, but they appear to be associated with the surface, probably the capsule, of the organism. The production of toxic substances by the meningococcus has been demonstrated by various workers (Branham, 1940); knowledge of these is, however, vague, and it has not been proved that they play any part in the passage of the organism to the subarachnoid space.

The low incidence of meningitis in spite of the wide distribution of the meningococcus indicates clearly that the passage from the nasopharynx to the meninges is not readily accomplished. An important reason for this is the barrier to invasion presented by the nasopharynx; the responsible factors consequently merit close consideration.

Resistance of the host.—During the 1939–45 war conditions favoured the wide dissemination of the meningococcus, particularly in the Services, as overcrowding was common and ventilation of rooms often inadequate as a result of stringent black-out precautions. Although there was a greatly increased incidence of meningitis during the main outbreak of 1940–42, the majority of cases were single and widely distributed, having no direct contact with each other. Most individuals would, therefore, appear to possess a high degree of resistance against the meningococcus, and the factors concerned in this have been the subject of much speculation.

Many advocate the theory that infection with the meningococcus occurs in three progressive stages: (1) catarrhal, (2) septicaemic, and (3) meningitic; the common form of the infection is a nasopharyngitis, and only in relatively few cases does the organism penetrate the pharyngeal barrier to reach the blood stream, from which the meninges are attacked. The nature of this pharyngeal barrier has, however, never been satisfactorily explained.

The so-called catarrhal stage is obviously the one of greatest epidemiological significance in view of the potential danger to the community. It is consequently important that the criteria on which this theory is postulated should be critically examined.

The main arguments put forward to support this theory are: (1) the isolation of the homologous meningococcus from both the nasopharynx and cerebrospinal fluid of early cases (Flack, 1917), (2) the history of premeningitic sore-throat or catarrh given by some cases, and (3) the widespread distribution of carriers.

These views have not passed unchallenged, and the following points have been raised in opposition: (1) the failure of many workers to isolate regularly the meningococcus from the nasopharynx of early cases of meningitis (Andrewes and others, 1916); it is also interesting to note in this connexion that Fildes and Baker (1918) reported the occurrence of meningitis in twenty-six naval ratings although their nasopharyngeal swabs had been negative at varying periods from two to seventy-five days before the onset of symptoms, and concluded that cases can seldom be carriers before the onset of the disease; (2) a history of sore-throat or catarrh is frequently unobtainable from cases or carriers (Foster and

Gaskell, 1916; Worster-Drought and Kennedy, 1919); (3) cerebrospinal meningitis occurs mainly during the early months of the year when upper respiratory infections are common, and their association with meningitis is likely to be coincidental (it has even been suggested that this non-specific catarrh might constitute a predisposing factor to the development of meningitis); (4) case-to-case infection is extremely rare; the source of infection is almost invariably an undetected carrier; (5) in the majority of instances the carrier state is temporary.

As a reply to these arguments protagonists of the catarrhal theory claim that the local meningococcal infection is generally mild and transient, giving rise to little or no personal discomfort, but clinical and bacteriological confirmation is seldom produced.

There is general agreement that in every case of meningitis the portal of entry of the meningococcus is the nasopharynx. There is, however, a lack of convincing evidence to indicate that the production of a nasopharyngitis, even in a mild form, is an essential stage in the development of meningitis. It must be pointed out that the examination of nasopharyngeal swabs from early cases is rarely carried out, and knowledge of this important aspect of the subject is consequently limited.

During this investigation the validity of the "catarrhal" theory was examined by the collection of swabs from the following groups of the community:

1. *Close contacts.*—These were soldiers sleeping in beds adjoining the case, or all occupants in cases arising in tents or billets. All had had ample opportunity of receiving the upper respiratory flora from the cases as overcrowding was often present and ventilation, particularly at night, usually poor; the swabs were collected within a short time, often a few hours, of the removal of the case.
2. *General community.*—Swabs were collected from persons having no direct contact with cases in order to obtain some indication of the general carrier rate. These have been sub-divided into two groups—one containing swabs collected at the time of the main outbreak (1940–42) and the other comprising those taken during an "interepidemic" period (1946–47).
3. *Cases of meningitis.*—Swabs were collected from 22 cases, in 16 of these within 48 hours of onset. Group I strains were isolated from the cerebrospinal fluid of 13 cases but no growth was obtained from the other 9, 3 of which were of the mild, abortive type. The nasopharyngeal swab from one of these mild cases gave a heavy growth of a Group II strain.
4. *Cases of chronic meningococcal septicaemia.*—Two only were investigated.

The results obtained from these swabs are presented collectively in Table III

TABLE III

THE ISOLATION OF THE MENINGOCOCCUS FROM NASOPHARYNGEAL SWABS

Group	Number examined	Number positive	Group I	Group II
Close contacts (1940–41)	145	21 (14%)	6 (4%)	15 (10%)
General community .. (epidemic period 1940–42)	410	75 (18%)	11 (3%)	64 (15%)
General community .. (non-epidemic period 1946–47)	120	8 (7%)	1 (1%)	7 (6%)
Cases of meningitis	22	6 (27%)	4 (18%)	2 (9%)
Cases of chronic septicaemia ..	2	1	0	1*

*A few colonies of a Group II meningococcus were isolated from the nasopharyngeal swab some 21 days after the onset of symptoms; a Group I strain was isolated by blood-culture.

These results provide little bacteriological evidence to support the "catarrhal" theory. Meningococci were isolated from the nasopharynx of only 6 of 22 cases and in the majority of these the organisms were not numerous; the 1940–42 outbreak was caused almost entirely by Group I strains, but during this period the majority of carriers harboured Group II strains; there was no significant difference in the carrier rates of Group I strains given by the close contacts (4 per cent) and the general community (3 per cent), in both the rates were low; few of the cases or carriers gave a definite history of sore throat or catarrh.

The meningococcus is transmitted by droplet infection, and therefore in all persons developing meningitis these organisms must have been present in the nasopharynx during the initial stages of the infection. Their subsequent isolation from this site is dependent not only on the interval between implantation of the organisms and the collection of the swab but also on the nature of the local reaction. If a local infection develops it is reasonable to expect (1) the isolation of meningococci from the nasopharyngeal swabs in many early cases—not infrequently in practically pure culture, and (2) a relatively high carrier rate of the infecting type of

organism among close contacts, as an interchange of the upper respiratory flora takes place readily among occupants of overcrowded and badly ventilated rooms. Such results have rarely been obtained.

It is recognized that a small number of carriers are of the "persistent" type and that the persistence of the carrier state is associated with some pharyngeal abnormality (Cleminson, 1918; Embleton and Steven, 1919). During this investigation persistent carriers were found to have some abnormality, such as enlarged adenoids, and the majority harboured Group II strains often in relatively pure culture. These organisms were usually associated with a chronic form of local infection, but they were of little epidemiological significance as the main outbreak was caused by Group I strains; they were readily cleared by a course of sulphonamides (Fairbrother, 1940).

The failure to detect the homologous meningococcus in swabs from the two chronic septicaemia cases is not surprising. Logan (1946), in an excellent review of meningococcal septicaemia, points out that the pathogenesis of the disease is uncertain; there is no bacteriological proof that the portal of entry of the organisms is the same in meningococcaemia as in meningitis, although this is probable, while a persistent focus of infection has seldom been found. In the two cases under review, the swabs were collected when the disease was well established and the negative results indicate only that the focus of the existing infection was not the nasopharynx.

Pathogenesis

The path of the meningococcus from the nasopharynx to the cerebrospinal fluid has not been conclusively determined, and in consequence it has been the subject of much speculation. Two main views have been formulated; the first accepts the direct extension of the meningococcus from the pharynx to the meninges, the second postulates that the organisms are transmitted by way of the blood stream.

The theory of direct extension was advocated during the 1914–18 outbreak but convincing proof was not produced. There has consequently been increasing support for the "haematogenous" theory, which is now generally accepted (Brinton, 1941; War Office Memorandum, 1942; Strong, 1943; Banks, 1947). It must, however, be appreciated that the available evidence to support this view is inconclusive and merits examination.

The main arguments advanced in favour of the "haematogenous" route are: (1) the development of a so-called premeningitic stage with involvement of the blood stream; (2) the rare isolation of the organism by blood-culture during the premeningitic phase while the cerebrospinal fluid was normal; (3) the isolation of the meningococcus by blood culture from early cases of meningitis; and (4) the occurrence of a septicaemic form of meningococcal infection in which there is no involvement of the meninges—these infections may occasionally be of a fulminating character.

It has already been pointed out that a satisfactory explanation has not been produced to indicate how the meningococcus enters the blood stream from the nasopharynx. Considerable doubt has, moreover, been cast on the "catarrhal" theory and there is little evidence to indicate that there is a persistent focus of infection in the nasopharynx from which the organisms pass into the blood. Also a satisfactory explanation of the method or route by which the meningococcus passes from the blood to the meninges has not been forthcoming. In this respect, it is interesting to note that there is a definite blood–central nervous system barrier; the passage of substances through the choroid plexus or other vessels into the cerebrospinal fluid is not automatic but is controlled by some form of selective mechanism. It is well known that penicillin injected into the blood does not readily reach the theca; while experimental infection with the poliomyelitis virus is extremely difficult to produce by the intravenous route, although it occurs readily by the intrathecal or intraneural route.

There is no evidence to suggest that the meningococcus has any special affinity for the choroid plexus or other part of the theca. It is indeed accepted that in most cases of meningococcal septicaemia the development of meningitis does not occur even though, in pre-sulphonamide days, the blood infection often persisted for months.

Moreover, a positive blood culture in the early and acute stages of a severe infection cannot be accepted as evidence of primary blood invasion. In such infections as pneumonia and enteric fever a positive blood culture is frequently present in the early stages as a secondary phenomenon, being an overflow of the organisms from the primary focus of the infection. It is, therefore, reasonable to claim that the positive blood culture obtained in many cases of meningococcal meningitis has been a secondary development.

A more serious argument is the rare occurrence of a positive blood culture before the development of meningitis; it must, however, be noted that in several of these cases the primary focus of infection was not established, the presence of a rhinopharyn-

gitis being assumed, and/or the presence of meningitis was not excluded by lumbar puncture.

The occurrence of a definite premeningitic clinical stage can also be challenged. Cases of moderate severity tend to give a twenty-four-hour history of general malaise, weakness, headache of varying but usually progressive intensity, and slight neck rigidity. On lumbar puncture a turbid fluid is almost invariably obtained from these cases; this is proof that the invasion of the meninges occurred many hours previously. In fulminating meningitic cases, a turbid fluid is obtained by lumbar puncture even though the history of the infection is short, usually a matter of hours; the possibility of such infections developing in progressive stages (viz., catarrhal and septicaemic) is remote.

There is no specific sign or symptom to indicate involvement of the meninges; the initial symptoms of moderate meningeal infections are often those of an indefinite character, and such cases are not infrequently diagnosed tentatively as influenza. The presence of meningitis can be established only by an examination of the cerebrospinal fluid. The development of a premeningitic phase of the infection cannot therefore be accepted on clinical grounds alone.

In order to test the "haematogenous" theory, blood cultures were prepared as early as possible in the infection from a number of cases, usually at the time of collection of the cerebrospinal fluid and nasopharyngeal swabs. The results are given in Table IV.

TABLE IV

ISOLATION OF THE MENINGOCOCCUS FROM THE BLOOD AND CEREBROSPINAL FLUID IN CASES OF MENINGITIS

Time of collection	No. examined	No. positive	C.S.F. positive in
Within 24 hours of onset	12	1	12
24–48 hours of onset	8	1	6
Later than 48 hours of onset	5	1	3
Total ..	25	3	21

The meningococcus was isolated from the blood stream in only 3 out of 25 cases (12 per cent). In 20 of the 25 cases the blood was collected within forty-eight hours of the onset of the disease; in all cases a well-established meningitis was present and meningococci were isolated from the cerebrospinal fluid of 21 of the 25.

These results are not consistent with the development of a premeningeal septicaemic stage, but indicate merely the secondary invasion of the blood stream from the primary infection of the meninges. A positive blood culture, obtained when meningitis is present and the cerebrospinal fluid contains large numbers of meningococci, cannot be accepted as evidence of either the passive transfer by the blood stream of the organisms to the subarachnoid space from an indefinite focus of infection in the nasopharynx or the presence of a distinct premeningeal phase.

It is interesting to note that the majority of positive blood-cultures from cases of cerebrospinal meningitis, as distinct from chronic septicaemia, reported in the literature have been obtained when the meningitis was well established.

Discussion

The results of this investigation do not provide evidence, clinical or bacteriological, to support the widely accepted theory that the meningococcus produces a primary pharyngeal catarrh, from which, in relatively few cases, it passes to the meninges by way of the blood stream.

This is not surprising, as evidence advanced to support this theory is far from conclusive. To account for the failure of many cases to provide a history of sore-throat or catarrh, it was suggested that the local pharyngeal infection was often transient and trivial, but bacteriological or clinical proof of this infection was seldom obtained. Moreover there has been no satisfactory explanation of the method by which the organisms enter the blood stream from such a trivial primary lesion or by which they subsequently pass from the blood stream to the meninges.

In view of the fundamental weaknesses of the haematogenous theory it is essential that an alternate method of spread, namely by direct extension, should be carefully examined. This theory received much support during the 1914–18 outbreak, when the following routes were suggested: (1) by the perineural lymphatics of the olfactory nerves through the cribriform plate of the ethmoid; (2) through the sphenoidal sinuses; (3) via the middle ear. Convincing evidence to support any of these routes was not produced, and this theory consequently became neglected.

In 1929, however, Clark produced conclusive proof that material could pass directly to the brain from the nasal passages. Investigating the development of nervous complications following vaccination, Clark showed that a solution of potassium

ferrocyanide and iron ammonium citrate reached the subarachnoid spaces of the brain of rabbits within one hour of being dropped into their nasal cavities. The route of spread was not by the lymphatics but by the perineural sheaths of the olfactory nerves, which provide continuity between the subarachnoid space and the olfactory sensory epithelium; in these spaces a centripetal flow was postulated. It was also found that some of the solution entered the blood capillaries. On treatment of the tissues, the deposit of Prussian blue in the nasal mucosa was found to be considerably more abundant in the olfactory area, which is innervated by the olfactory nerves, than in the "respiratory" portion which is not reached by the olfactory nerves. Clark considered that a similar sequence of events, apart from variations in degree of absorption from the mucosa, was probable in man, and suggested that these findings had an important bearing on the passage of infective material from the nasal cavities to the brain.

It is also interesting to note that, during experimental work on poliomyelitis, Faber (1933) demonstrated that the hairs of the olfactory nerves lie free in the olfactory portion of the nasal mucosa and are covered only by mucus. In spite of this it is important to note that intranasal application of the poliomyelitis virus into the nares of monkeys does not invariably lead to the development of experimental infection even though the monkey is a relatively susceptible animal (Fairbrother and Hurst, 1930). The poliomyelitis virus passes to the central nervous sytem along the axons of the olfactory nerve. This is a different route from that described above, but the area of nasal mucosa at which the neural tract is entered is probably the same in both cases.

There is thus reliable evidence of definite but complex communications between the nasal cavities and the central nervous system; these are situated in the olfactory portion of the nasal mucosa and occupy a relatively small area on the septal and lateral walls of the superior nasal meatus. Passage of infective material along the olfactory tract is not, however, readily accomplished.

Applying these observations to the pathogenesis of meningococcal meningitis, the following hypothesis is postulated.

The meningococcus is widely disseminated by droplet infection and gains entrance to the upper respiratory tract by the nostrils and mouth. The subsequent sequence of events is dependent on many factors, particularly important being the virulence and the number of the organisms, their implantation on the olfactory portion of the nasal mucosa, and the subsequent overpowering of the local defence mechanism.

In the majority of individuals these requirements are not fulfilled; the meningococcus becomes a transient member of the pharyngeal flora, probably as a commensal, and the individual becomes a temporary carrier. Under circumstances particularly favourable to the meningococcus, the local defence barrier is overcome and the organisms may, by direct extension, reach either the theca or possibly the blood capillaries; in the latter event the septicaemic type of infection might result.

Many factors are concerned with the tendency for outbreaks to appear under war-time conditions. During the war 1939–45 opportunities for the dissemination of the meningococcus were greatly increased as a result of overcrowding and poor ventilation, which tended to be accentuated by black-out precautions, while in certain groups, such as new recruits, general resistance was probably lowered by fatigue and exposure, especially during the winter. It is also interesting to note that Group I strains were responsible for the main outbreak and there is much indirect evidence to indicate that these strains are more virulent than Group II.

A possible explanation for occurrence of sporadic Group II infections, found mainly in infants and children, during inter-epidemic periods is the close contact of infants with adults, in particular mothers and nurses, and the consequent greater risk of frequent and large dosage with these strains. Adults may possess some form of specific resistance to the meningococcus, but there is no convincing evidence that the development of specific immunity plays a major role in the defence against meningococcal infections. It is, however, possible that the local defence mechanism is less efficient in infants than in adults, and is more readily overpowered by the relatively avirulent Group II strains.

Summary

The results of this investigation do not support the theory that meningococcal infections occur in three successive stages—catarrhal, septicaemic, and meningitic. Support is given to the theory of direct spread of infection from the nasopharynx to the theca by the perineural sheaths of the olfactory nerves.

I am indebted to many colleagues, in particular Dr. D. C. Liddle, for assistance in the collection of material for this investigation, and wish to offer my sincere thanks for their help.

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RETICULOCYTES AND THEIR HUMORAL REGULATION

BY

ERIK JACOBSEN

From the Biological Laboratories of Medicinalco Ltd., Copenhagen

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The reticulocytes are generally accepted to be young red blood cells. Whenever red cell formation in the bone marrow is active the proportion of reticulocytes in the blood increases; hence the importance of the reticulocyte count in haematology. Nevertheless, there have been few studies dealing with purely physiological and chemical aspects. A study of the ripening of reticulocytes into mature non-reticulated red cells has, however, been carried out in our laboratory during the last five or six years. The present article reviews the results obtained.

Experimental Procedure

Rabbits were used as experimental animals. In order to obtain reasonable accuracy in counting their reticulocytes, the animals were brought into a chronic anaemic state by the daily withdrawal of from 30 to 50 ml. of blood. At the end of one week the haemoglobin percentage thus fell to 40 or 50 and the red cell count to 2 or 3 million per c.mm. of blood, with 20 or 30 per cent reticulocytes. This state could be maintained for a month or more before the animals died. The blood of such animals was used in the tests (Plum, 1942a).

Blood was centrifuged and the red cells washed in saline. They were then re-suspended in saline, or in plasma or saline to which had been added the substance whose action on the reticulocytes was to be studied. The suspensions were kept in small test-tubes in a water bath at 40° C. and gently and constantly moved. Samples for reticulocyte counts were taken at regular intervals.

Reticulocytes suspended in saline alone disappeared slowly, but the rate of disappearance could be accelerated considerably by adding commercial liver extract to the saline in which the cells were suspended (Fig. 1). The youngest and most unripe types of reticulocytes disappeared first, and as there was apparently no haemolysis this must have been due to their maturation into "adult" red cells. This process seemed to be accelerated by some substance found in liver extracts.

The ripening process followed the monomolecular equation well known in physical chemistry and biochemistry:

$$k = \frac{1}{t} \log \frac{a}{a - x}$$

where t is the time of incubation, a the number of reticulocytes at the beginning of the experiment, and x the number of reticulocytes that disappear during the time t; k is the "monomolecular constant." This formula only indicates that the same percentage of reticulocytes is ripened in the same interval of time regardless of the number of reticulocytes. The constant, however, gives a very convenient measure for the rate of ripening; the higher the constant, the faster the rate, and vice versa; k increases with rising temperature.

Experiments with varying concentrations of liver extract have shown that the constant in the ripening

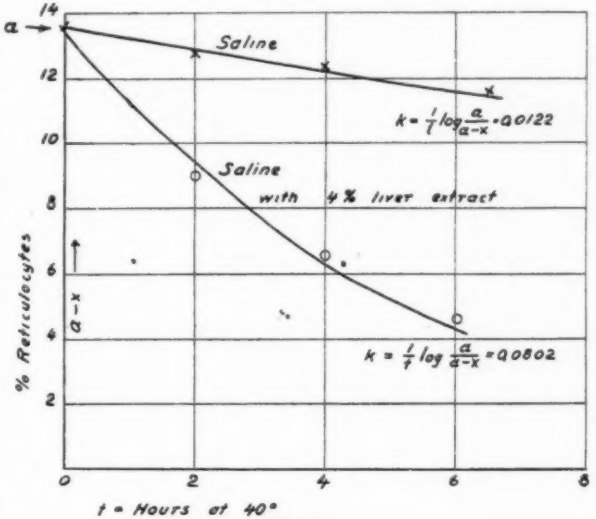


FIG 1.

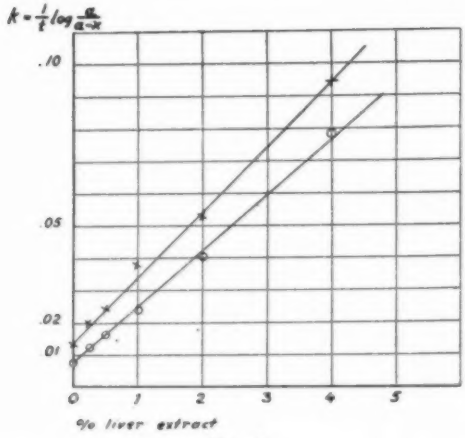


FIG. 2

experiments is directly proportional to the concentration of liver extract (Fig. 2).

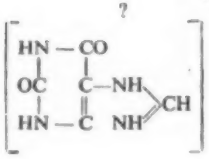
Chemical Nature of Reticulocyte-ripening Principle

The reticulocyte-ripening principle in liver extract is thermolabile; it loses its activity after heating for five minutes on a boiling water bath. Thus it cannot be identical with the thermostable principle acting against pernicious anaemia. It has been shown, moreover, that the reticulocyte-ripening principle in liver consists of a thermolabile fraction which can be absorbed to floridine and a thermostable fraction that cannot be absorbed (Jacobsen and Plum, 1942). The latter fraction has little effect by itself, but activates the effect of the thermolabile component. The thermostable fraction can be extracted from liver extract with butanol and isolated from the butanol extract. It has been

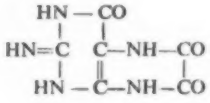
TABLE I

Reticulocyte ripening principle

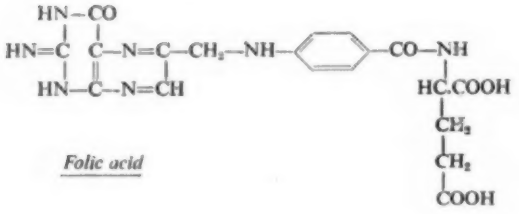
thermolabile fraction



Xanthine

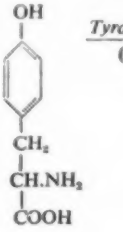


Leucopterin

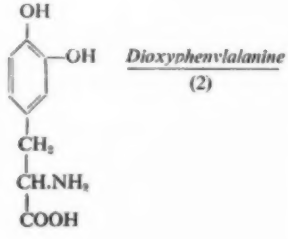


Folic acid

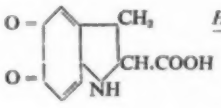
thermostable fraction



Tyrosine (1)



Dioxyphenylalanine (2)



Hallachrom (50)

found to be identical with l-tyrosine. Synthetically prepared tyrosine shows exactly the same effect in combination with the thermolabile fraction.

The effect of tyrosine seems to be specific. None of the thirteen most common amino acids, including phenylalanine, has any activating effect on reticulocyte ripening, but compounds similar to tyrosine, such as adrenaline and tyramine, have some effect (Jacobsen and Plum, 1942). Later investigations have shown that certain oxygenated products of tyrosine, dioxyphenylalanine and hallachrom, have a much stronger activating effect, that of hallachrom being 50 to 100 times stronger than that of tyrosine (Gad and others, 1944). As there are enzymes present in red cells capable of converting tyrosine into hallachrom it is possible that the activating effect is not due to tyrosine, but to hallachrom derived from it.

The thermolabile reticulocyte-ripening fraction has not been isolated from liver. Systematic investigations on various organs have shown, however, that stomach tissue (Plum, 1944a), duodenum, and the upper part of the small intestine contain considerable amounts of the thermolabile fraction (Bohn, 1946). Extracts from these tissues have little ripening activity by themselves, but marked ripening effects, even greater than that of liver extracts, have been found after the addition of tyrosine, dioxyphenylalanine, or hallachrom. The ripening fraction in gastric tissue is extremely labile; however, a rather powerful preparation containing 75 to 80 per cent xanthine has been isolated from it (Jacobsen, 1944). Synthetically prepared xanthine in combination with tyrosine has a marked effect, but it does not seem likely that the effect of the gastric extract can be explained by its content of xanthine. Probably a tautomeric isomer is responsible. Some chemical analogues of xanthine have been tested. Guanine and caffeine amongst others are ineffective. Recently both leucopterin and folic acid have been shown to be more potent than xanthine, although with qualitative differences. Xanthine is only effective in the presence of tyrosine and is thermolabile. Leucopterin and folic acid have, however, an effect even without tyrosine, but are activated 100 per cent or more after its addition. The potency of neither of these compounds is affected by boiling.

The chemical problem has not been solved. Several experiments indicate that the thermolabile fraction consists of more than one substance. The fact that substances which have recently been shown to have an effect on haemopoiesis *in vivo* may accelerate ripening of reticulocytes *in vitro* is, nevertheless, of great interest.

Content of Reticulocyte-ripening Factors in Organs and Plasma

As has already been stated, reticulocyte-ripening principles may be found in extracts from several organs of the body. Calculated per gramme of tissue, the greatest concentration is found in plasma, and slightly less in bone marrow, liver, stomach, and spleen (Plum, 1944a). Extracts of all these organs, but not plasma, may be activated by the addition of tyrosine (Fig. 3). The stomach and the upper intestinal tract contain the highest concentration of the thermolabile fraction. Large amounts are found in the duodenum and in the upper part of jejunum, apparently following the distribution of Brunner's glands. The results of chemical investigations on stomach extracts have already been mentioned. In Fig. 3 is shown the distribution in swine ; similar relative distributions are found in the organs of oxen, rats, rabbits, and guinea-pigs.

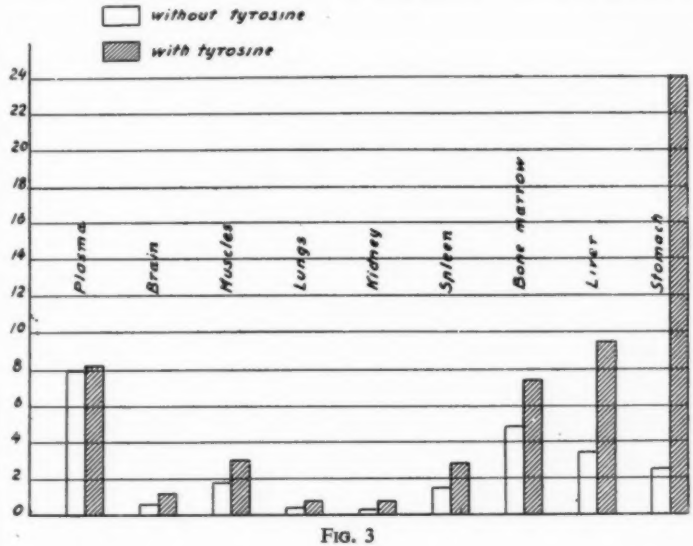


FIG. 3

B

Unlike liver extracts the reticulocyte-ripening principle in plasma cannot be activated by tyrosine, and plasma is not able to activate the thermolabile fraction further as it ought to if it contained tyrosine or tyrosine-like substances. However, when plasma is treated with acetone, the ripening principle is transformed into a state in which it can be activated by tyrosine. This shows that there is no great difference between the ripening principles in plasma and liver; the linkage between thermolabile and thermostable fractions in plasma must be a strong one.

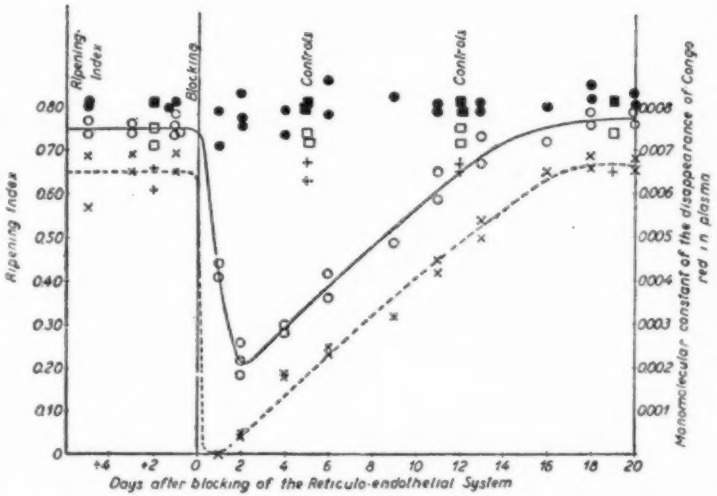


FIG. 4

ROLE OF THE RETICULO-ENDOTHELIAL SYSTEM

Experiments with rabbits have shown that the action of the thermostable component is influenced by the activity of the reticulo-endothelial system. The content of ripening substances in plasma has been determined with and without the addition of tyrosine. After a control period the reticulo-endothelial system was blocked by intravenous injection of 10 ml. 1 per cent trypan blue combined with splenectomy. The activity of the reticulo-endothelial system, as tested for by the disappearance of congo red, rapidly drops to zero and recovers slowly, becoming normal again by the sixteenth to eighteenth day. Fig. 4 demonstrates that the amount of ripening principle in plasma closely follows the activity of the reticulo-endothelial system; after addition of tyrosine, however, there is no difference from the normal. Blocking has thus no influence upon the thermolabile component.

These findings make it seem probable that tyrosine (or a derivative of, or an oxygenated product of, tyrosine) and the thermolabile substance are linked in the reticulo-endothelial system and together form the reticulocyte-ripening principle found in plasma (Jacobsen and Plum, 1943a).

Content of Reticulocyte-ripening Principles in the Plasma of Various Animals and Man

As the content of the ripening factors in organs varies with that in plasma, most of the investigations

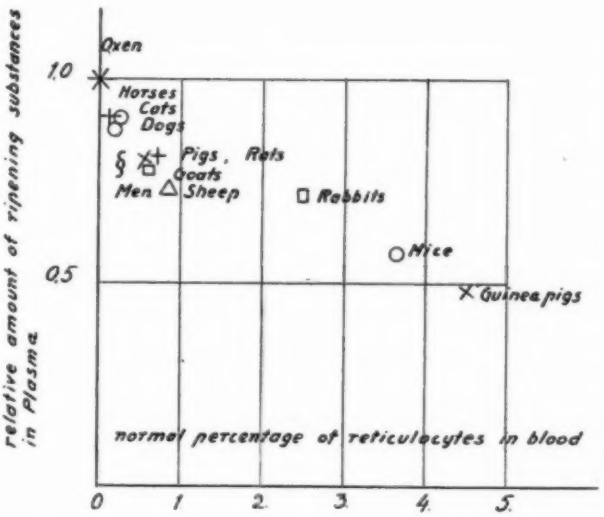


FIG. 5

have been carried out on the plasma of various animals. The amount of reticulocyte-ripening factor in plasma varies in different animals (Plum 1942b, 1943). Reptiles and birds have practically none. In mammals there is considerable variation (Fig. 5). The plasma of animals whose blood normally contains few or no reticulocytes (for example, oxen) seems to contain the highest content of ripening substances, whilst that of species whose blood normally has a relatively high percentage of reticulocytes (e.g., guinea-pigs) has the lowest content, in the case of guinea-pigs half that of oxen.

The content of ripening substances in the plasma of normal adults also seems to vary inversely with the reticulocyte count. In certain physiological and pathological states, however, this relationship may not hold (Table II).

TABLE II

	Reticulocytes	Ripening substances
Early life	+++	+++
Anaemia due to loss of erythrocytes	++	+
Iron deficiency	+	+
Benzol poisoning	+	+
Gastrectomy or duodenectomy	(+)	—
Pernicious anaemia ..	?	—
Hypothyroidism	0	—
Hyperthyroidism		—
Hypophysectomy		—
Menstruation	(+)	(+)
Jaundice, obstructive and haemolytic	(+)	(+)

In foetal life and in infancy until the blood picture becomes normal, the increased numbers of reticulocytes are associated with a considerably increased amount of ripening principles. In newborn rats or rabbits with 80 or 100 per cent of their red cells in the form of reticulocytes, about double the normal concentration of ripening substances may be found. Both reticulocyte percentage and content of ripening substances decrease gradually and reach normal values at the same age (Plum, 1943). A probable explanation of the occurrence of high reticulocyte percentages and high concentrations of ripening substances, for which there is experimental support, is that the immature blood corpuscles in the early ages react more slowly to humoral stimulation (Jacobsen and Plum, 1943b).

The reticulocytosis associated with increased blood formation, as seen in chronic post-haemorrhagic anaemia in rabbits, is always combined with an increase in ripening substances (Plum, 1943). In this case the response of the reticulocytes to a concentration of ripening substances does not change, but the quantity of ripening substances in the plasma increases. This increase may cause the reticulocytes in the peripheral blood stream of anaemic rabbits to ripen about 20 or 30 per cent more rapidly than in normal animals. The same phenomenon is seen when anaemia is caused by some haemolytic poison such as phenylhydrazine (Plum, 1944b).

Similarly, in iron-deficiency anaemia of dietary origin reticulocytosis is accompanied by an increased concentration of reticulocyte-ripening substances (Plum, 1944b). On treatment with iron, both anaemia and reticulocytosis disappear and the content of reticulocyte-ripening substances in the plasma returns to normal. Anaemia and leucopenia result from the effects of benzol poisoning on the bone marrow. The reticulocyte count may fall or rise slightly, but the content of reticulocyte-ripening substances in the plasma is always increased. During regeneration with increases in leucocytes and reticulocytes the content of ripening substances returns to normal. These experiments suggest that the rise in reticulocyte-ripening substances may be interpreted as one of the measures by which the body attempts to compensate for anaemia.

Occasionally an increased number of reticulocytes is associated with reduced amounts of reticulocyte-ripening principles in the plasma, as in rats after gastrectomy (unpublished observations) and in swine in which the duodenum had been either isolated or excised causing a 20 per cent decrease in activity (Bohn and others, 1945).

In untreated pernicious anaemia a decrease up to 40 per cent has been observed. The addition of tyrosine has in all cases raised the concentration of ripening substances up to the normal level. This decrease is thus not due to a decreased concentration of the thermolabile component, as might have been expected from the known high concentration of the thermolabile fraction in the gastric tissue of the normal subject and the characteristic gastric atrophy in pernicious anaemia.

Both in spontaneous hypo- and hyperthyroidism in man and in the experimental animal there is a decreased content of ripening substances in the plasma, which may be corrected by the addition of tyrosine. The decrease after hypophysectomy may result from decrease in the function of the thyroid gland.

During menstruation a slight increase has been observed starting on the first day.

Obstructive jaundice in patients and in animals may be associated with a slight increase in ripening substances.

Conclusion

Assay of the concentration of reticulocyte-ripening substances in the plasma is of more scientific than practical interest. It has no diagnostic value. The plasma of from one hundred to one hundred and fifty patients with various diseases and that of about fifty normal persons have been tested. A definite decrease in the content of ripening substances in the plasma has been found in untreated pernicious anaemia, in Graves's disease, in some patients with gastric diseases, and in cachectic patients suffering from cancer (Plum, R., 1947). In all cases this decrease has been due to lack of the thermostable fraction and could be rectified by the addition of tyrosine. In some patients with jaundice the content of the ripening substances has been increased. In all other diseases normal levels have been found.

Even a slight reticulocytosis has been generally considered a sign of increased erythropoiesis. The work now described indicates that this may not always be the case. The reticulocyte percentage in peripheral blood depends upon the output of reticulocytes from the bone marrow, controlled by factors still unknown, and the rate of ripening in the blood stream, the latter regulated by the ripening principles. Thus with a constant production the numbers of reticulocytes in the circulating blood can increase if the concentration of ripening substances in plasma decreases, and vice versa. Slight reticulocytosis cannot, therefore, be regarded as a sign of increased or altered blood production unless it is certain that the concentration of ripening substances has not altered.

It is difficult to make any definite statement with regard to the physiological role of the ripening substances, particularly as it is doubtful what reticulocytes really are. It is thought that under normal conditions not all the red cells released from the bone marrow are in the form of reticulocytes. As the capacity of the reticulocytes to transport oxygen is as good as that of adult cells, it is doubtful whether their maturation into adult corpuscles is of any great physiological significance. The as yet unpublished observations of my collaborator, Dr. C. M. Plum, who has done a major part of the work described in this paper, seem to show that a similar mechanism may operate in the formation of red cells from normoblasts. If this is true, the ripening of reticulocytes can be regarded as a special case or model of the course of erythropoiesis as a whole. The demonstration of a humoral regulation of blood-cell formation may be expected to throw new light on many of the hitherto unsolved problems in erythropoiesis.

Summary

A principle capable of accelerating the ripening of reticulocytes *in vitro* can be demonstrated in plasma and in various tissues of the body.

This principle consists of at least two fractions: a thermostable one, identified as tyrosine or tyrosine derivatives, and a thermolabile one. Xanthine, leucopterin, and folic acid are able to act as the thermolabile factor. The greatest concentration of the thermolabile fraction is found in the stomach and duodenum. The thermolabile and thermostable fractions linked together by the activity of the reticulo-endothelial system form the principle found in plasma.

An increased amount of ripening principle may be found in the plasma in some cases of increased erythropoiesis; in decreased erythropoiesis a lower content than normal may be encountered. It seems as if the number of reticulocytes in the blood varies inversely with the amount of ripening principle, at least under normal conditions.

The significance of these findings is discussed.

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THE DISTRIBUTION OF LEUCOCYTES ON THE COUNTING CHAMBER

BY

MARTIN HYNES

From the Department of Medicine, University of Cambridge

(RECEIVED FOR PUBLICATION, JULY, 1947)

It is almost a statistical truism that the number of cells on each square of a haemocytometer is distributed in a Poisson series, but in fact it is easy to show that the distribution departs considerably from the theoretical expectation. The reason, perhaps, is that the experimental work confirming this theory was done on the old well type of haemocytometer, whereas the Bürker counting chamber is now universally used, and adds new and less predictable variations to random sampling.

My attention was drawn to this point by a statistically controlled series of leucocyte counts on Bürker counting chambers with the Neubauer ruling. I counted, as is usual, the number of cells on four areas of one square millimetre at the corners of a square with a side of three millimetres, and recorded the four sub-totals separately. Over the whole series, the two squares nearest to the filling point of the chamber gave nearly identical totals 3.5 per cent below the mean value for all four squares, and the two further squares similarly gave nearly identical totals 3.5 per cent above the mean value. The difference was statistically highly significant. It seemed worth while to test the extent of this deviation from the theoretical Poisson distribution, and to see whether it might be a source of error in blood counts.

Methods

The Bürker counting chambers used were by several different manufacturers, but all had some variety of Neubauer ruling. The cover-slips were from 0.4 to 0.5 mm. thick, and their position on the chambers was fixed by reference to permanent marks.

Blood from a finger-prick was diluted 1/10 in a leucocyte pipette with 2 per cent aqueous acetic acid coloured with 50 mg. of crystal violet per 100 ml. The contents of the pipette were mixed by vigorous shaking for two and a half minutes, half the contents were rejected, and the counting chamber was filled with care to avoid bubbles and over-filling. The chamber stood on a perfectly flat surface until the leucocytes had settled.

Areas of 0.1 sq. mm. on the counting chamber were defined by means of an eye-piece graticule and a $\frac{1}{6}$-inch objective, and the mechanical-stage vernier was used to set the fields of observation in rows 1 mm. apart across the length of the chamber. In each row the cells were counted in nine fields with their centres 0.5 mm. apart. In this way the whole filled area of the counting chamber was covered by a grid of observations with its boundaries 1 mm. from the edges of the cover-slip and the troughs.

The numbers of leucocytes observed in the grid of areas was entered into a table of equidistant columns along the length of the chamber, and of equidistant rows running across the chamber. The totals for the nine columns over a large series of separate counts did not differ significantly, that is, the side-to-side variation in the leucocyte distribution did not differ from expectation. It was, therefore, possible to measure lengthwise variation in the leucocyte distribution simply by reference to the "row" totals, which were large enough to prevent the Poisson distribution from interfering with the analysis of variance.

With each chamber and length of cover-slip the counts were repeated until 500 to 800 cells had been counted in each position along the length of the chamber. The "row" totals in each count formed the basis of the statistical analysis. In all, some 150,000 leucocytes were counted.

Single-cell Counting Chambers

A series of leucocyte counts was made on single-cell counting chambers fitted with cover-slips 16 mm. long in the longitudinal axis of the chamber. In

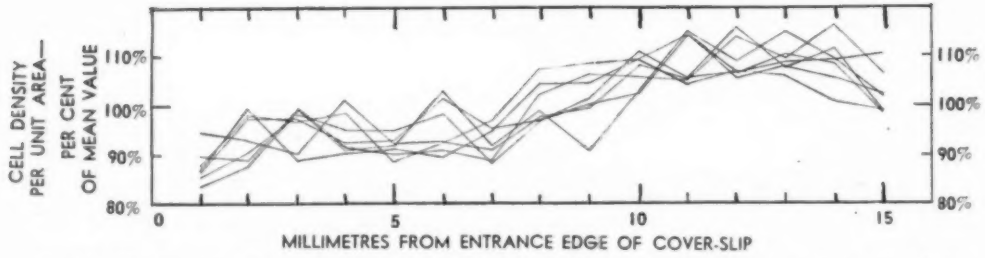

FIG. 1.—The leucocyte distribution on seven single-cell counting chambers. About ten counts were done on each chamber.

Fig. 1 each "curve" represents the total of nine or ten separate counts on seven counting chambers, each with its own cover-slip. Considerable random variation is to be expected in cell counts of the order of 500, but in spite of this a very definite and constant trend in the leucocyte distribution is evident. The statistical analysis of these figures is summarized in the Table as an example of the methods used throughout the investigation.

TABLE

THE ANALYSIS OF VARIANCE OF THE LEUCOCYTE TOTAL IN FIFTEEN POSITIONS ON THE COUNTING CHAMBER IN SIXTY-NINE BLOOD COUNTS

	Degrees of freedom	Sum of squares	Mean square	Variance ratio
Between bloods	68	226,164.87	3,325.95	48.0***
Between places	14	18,424.61	1,316.04	19.0***
Residue ..	952	65,893.69	69.216	
Total ..	1,034	310,483.17		
Between places	14	18,424.61	1,316.04	19.0***
Linear (ξ_1)	1	13,723.40		
Remainder	13	4,701.21	361.63	5.22***
Quadratic (ξ_2)	1	273.23		
Remainder	12	4,427.98	369.00	5.33***
Cubic (ξ_3)	1	2,049.92		
Remainder	11	2,378.06	216.19	3.13***
Quartic (ξ_4)	1	1,344.89		
Remainder	10	1,033.17	103.32	1.49
Quintic (ξ_5)	1	423.30		
Remainder	9	609.87	67.76	

The analysis of variance is first divided between "bloods"—due to the different leucocyte contents of the 69 specimens of blood used—and between "places"—representing the varying density of leucocyte distribution along the length of the counting chamber. The residual variance is a measure of experimental error, and is made up mostly of the component due to random sampling from a basically Poisson distribution. (The mean "row" total was 57.939.)

The significance of the difference between places is beyond question, so we next determine whether the variation in leucocyte density along the counting chamber can be represented by a straight line, or whether a more complex curve is necessary. This point is tested by fitting successive orthogonal polynomials (Fisher and Yates, 1943). The second half of the analysis in the Table shows how the calculation of each successive term removes a part of the sum of squares with a degree of freedom, until the remaining variance is not significantly greater than the residual variance. An alternative comparison is between the mean square from each term of the polynomials and the corresponding remainder mean square. This method gives the following values for the variance ratios:

ξ_1 38.0*** ξ_2 subnormal ξ_3 9.48* ξ_4 13.01** ξ_5 6.25*.

All the terms except the quadratic are significant.

The conclusion is that the variation in leucocyte distribution along the counting chamber cannot be represented by a straight line, but demands a quartic polynomial. This is a curve with a maximum towards either end of the cover-slip, as is shown in the subsequent figures.

It has often been surmised that the lines of the ruled area disturb the cell distribution on the counting chamber, but no significant effect was demonstrated in these observations. Fig. 2 contrasts the leucocyte distribution when the ruled area was under the centre of the cover-slip and when it was near to either end. The difference between the three curves is no greater than would be expected from random variation.

The total length of counting-chamber under the cover-slip has a considerable effect on the shape of the leucocyte distribution curve (Fig. 3), but all the curves have one important common property—that the leucocyte density reaches its mean value half-way along the cover-slip. If, therefore, the ruled area does not lie exactly under the centre of the cover-slip, the count will tend to be either too high or too low. The curves further show that the longer the cover-slip the less is the error from the ruled area not being central. If the ruled area lies 1 mm. away from the centre of the cover-slip, the error introduced is 4 per cent if the cover-slip is 11 mm. long, 3 per cent if it is 16 mm. long, and 2 per cent if it is 26 mm. long.

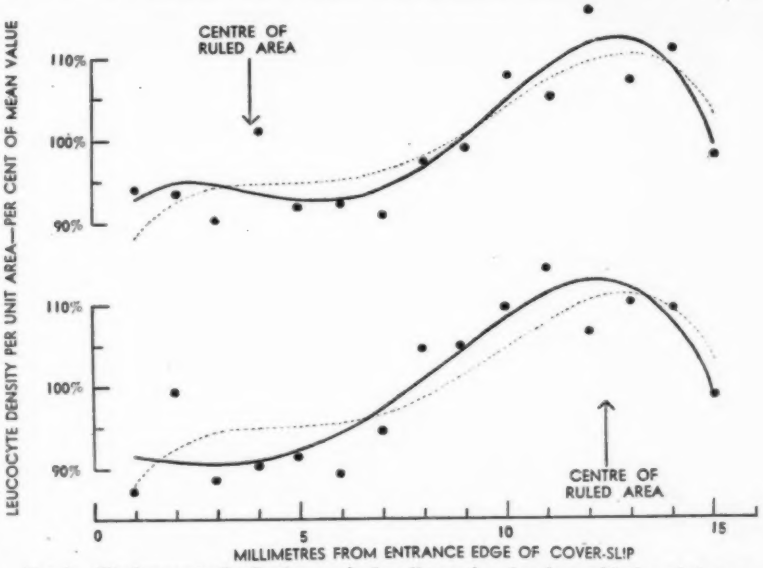

FIG. 2.—The leucocyte distribution on single-cell counting chambers with the ruled area under the centre of the cover-slip (broken line) or towards either end (continuous lines). The curves are the best-fitting quartics; the dots show the observed values relating to the continuous lines.

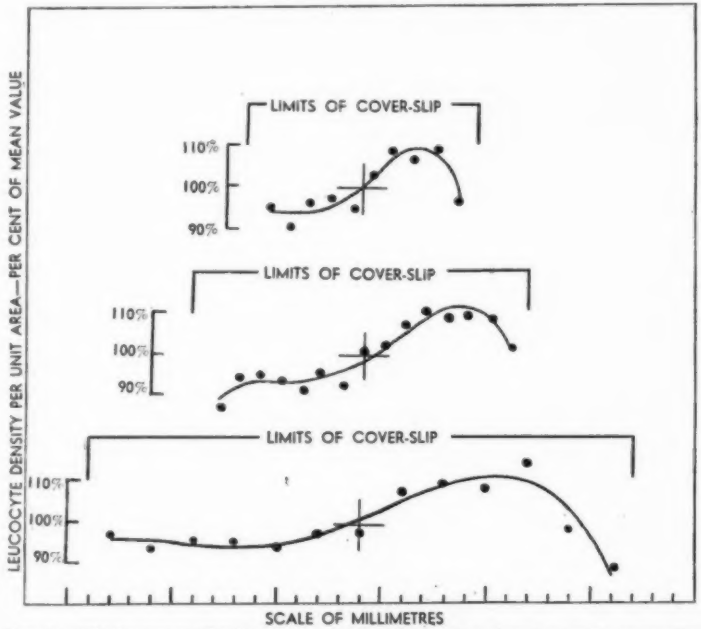

FIG. 3.—The leucocyte distribution on single-cell counting chambers with cover-slip of different lengths. The dots mark the observed values; the curves are the best-fitting quartics. The crosses mark the centre of the cover-slip and the mean value.

Double-cell Counting Chambers

The leucocyte-distribution on double-cell counting chambers (with a central trough and two ruled areas) varies in a very similar fashion (Fig. 4). Again the rule holds that the leucocyte density reaches its mean half-way along the cover-slip. The cover-slip should therefore be so placed that its edge and the edge of the central trough are equidistant from the centre of the ruled area. This is easily ensured by a suitable

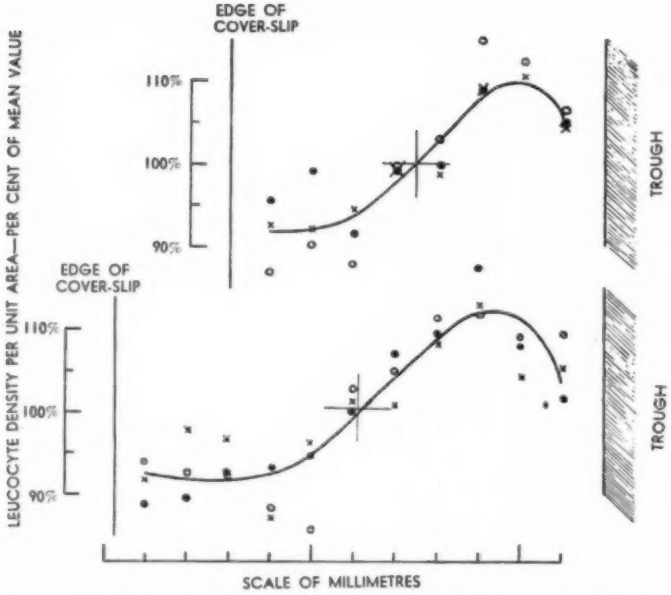

FIG. 4.—The leucocyte distribution on one side of double-cell counting chambers. The observed values from twelve counts on each of three counting-chambers with two cover-slips lengths are marked; the curves are the best-fitting quartics to the whole series. The crosses mark the mean value and the centre of the filled area of the cell.

mark when only one side of the counting chamber is in use, but if both sides are used simultaneously the cover-slip itself must be of the proper size.

The ruled area should be as far as is reasonably practicable from the central trough (5 mm. is a suitable distance), for the longer the area under the cover-slip the less is the slope of the leucocyte-distribution curve. Thus if the area covered is 9 mm. long, a displacement of 1 mm. of the cover-slip from "true centre" will lead to an error of 4.5 per cent, whilst if the area is 12 mm. long a displacement of 1 mm. will lead to an error of only 2 per cent.

Much greater errors may be introduced if no attempt is made to position the cover-slip. Some counting chambers have the centre of the ruled area only 3 mm. from the central trough; if 12 mm. of the cell in such a chamber were filled the leucocyte count would be nearly 12 per cent too high.

Reasons for the Effect

It is very unlikely that this variation in the leucocyte density on the counting chamber is due to bending of the standard cover-slips under capillary tension. A bending of this sort should produce a symmetrical distortion of the leucocyte distribution, rather than the asymmetrical distortion demonstrated, and different cover-slips would not produce such very similar effects.

The possibility that the uneven leucocyte distribution arose in the capillary stem of the pipette was also excluded. A 1/10 dilution of blood in leucocyte-counting fluid was made in small tubes containing glass beads, and after thorough mixing a drop of the fluid was transferred to a counting chamber with a platinum loop. It is not possible by this technique to fill the counting chamber with real accuracy, but the leucocyte distribution on the counting chamber agreed reasonably well with that obtained by the ordinary method (Fig. 5).

The true explanation of the uneven leucocyte distribution on the counting chamber seems to be the drift of the leucocytes along the chamber. When

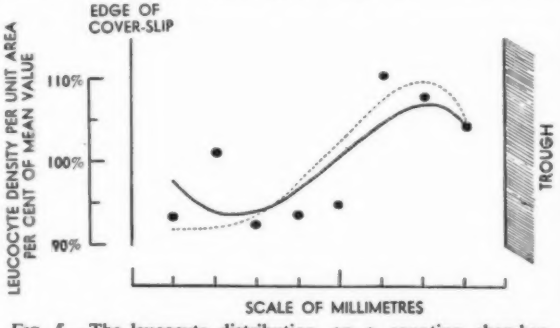

FIG. 5.—The leucocyte distribution on a counting chamber filled from a leucocyte pipette (broken line) and by the "tube and loop" technique (continuous line). The curves are the best-fitting quartics; the dots mark the observed values relating to the continuous line.

the chamber is filled, the leucocytes, having a greater density than the diluting fluid, will continue to flow forward after the fluid has stopped. The higher above the surface of the counting chamber a cell is when filling is completed, the further along the chamber the cell will float before it settles. Thus the nearer end of the chamber will lose leucocytes which will concentrate towards the further end.

This initial impetus of the leucocytes must soon be lost, but observation shows that any floating leucocyte is usually moving along the longitudinal axis of the counting chamber. This movement, which is probably engendered by convection currents as the fluid dries, is most marked at the two ends of the cover-slip, and is in the direction of the closer edge. Thus the secondary leucocyte drift tends to remove leucocytes from the middle of the cover-slip and to deposit them towards either end, so that the leucocyte-distribution curve has the two maxima shown above.

Floating leucocytes very rarely show any appreciable lateral drift, so that in any narrow segment across the chamber the leucocyte distribution obeys the Poisson law.

Discussion

It is clear that considerable errors can be introduced into a leucocyte count if account is not taken of the variation in leucocyte density along the counting chamber. The count may be as much as 10 per cent too low if cells are counted in an area between the "entrance edge" of the cover-slip and the centre of the filled area, or 10 per cent too high if cells are counted beyond the centre. The true leucocyte count is obtained only if cells are counted in areas symmetrically disposed about the centre of the filled area.

The leucocyte distribution curve has a steep gradient in the middle third of the filled area, but the steepness decreases as the length of counting chamber filled increases. With a length of 9 mm. the leucocyte density per unit area changed at the rate of 5 per cent per mm. at the centre of the filled area, whereas with a length of 26 mm. the rate of change was only 2 per cent per mm.

Thus the longer the area of counting chamber filled, the less will be the error from small inaccuracies in placing the cover-slip.

We should therefore make some new rules for blood-counts. With single-cell chambers the cover-slip should cover as much as possible of the chamber, and should be so placed that the ruled area lies exactly under its centre. With double-cell chambers, since both sides will normally be used together, the cover-slip should be of such a length that both ruled areas lie midway between an edge of the cover-slip and the nearer edge of the central trough. The ruled area should be as far as possible from the central trough, so long as it can still be centred under the cover-slip. Finally, when the cover-slip has been properly placed it is still necessary to remember the steep change in leucocyte density across the ruled area, and to count squares symmetrically disposed about the centre.

It is probably not possible to use the leucocyte-distribution curves obtained above to obtain a precise correction factor when the ruled area is not truly central. The central position of the mean value seems to be constant, but the shape of the curves must be influenced by many factors such as the viscosity of the diluting fluid and the rate of evaporation.

It seems, from a limited number of experiments, that the red-cell distribution on the counting chamber varies in a similar way to the leucocyte distribution. The slope of the curve is less, but the rule holds that the mean value is reached in the centre of the filled area.

Summary

1. The density of leucocytes per unit area on the counting chamber increases progressively from the point of entrance of the fluid along the length of the chamber.

2. The cell distribution in a narrow segment across the chamber obeys the Poisson law.

3. The curve of the leucocyte distribution along the chamber has two maxima, and statistically is adequately represented by a quartic polynomial.

4. The position of the ruled area does not affect the shape of the leucocyte-distribution curve.

5. The mean value for the leucocyte density per unit area is reached at the centre of the filled area of the chamber.

6. The rate of change in the leucocyte density is less the longer the filled area of the counting chamber.

7. The variation in the leucocyte distribution is not due to bending of the cover-slip, nor to any change in the cell distribution in the pipette before the chamber is filled. It appears to be due to the drift of the leucocytes caused by their initial forward impetus and subsequent convection currents.

8. Recommendations are made to counteract errors due to variation in the leucocyte distribution.

9. The distribution of red cells on the counting chamber follows similar laws to the distribution of leucocytes.

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THE COLORIMETRIC DETERMINATION OF GLUCOSE

BY

E. J. KING AND R. J. GARNER

British Postgraduate Medical School, London

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Several new descriptions of colorimetric methods for the determination of blood sugar have been described in recent years (for example, Polis and Sortwell, 1946; Somogyi, 1945; Nelson, 1944; Haslewood and Strookman, 1939). These procedures are all based on the use of alkaline copper reagents and on the production of molybdenum blue by the action of cuprous copper on complex molybdic acid solutions. With a view to finding the method or combination of methods which gives the most reliable results, particularly in the very low range of blood sugar, the following combinations of reagents were compared: (*a*) Harding's Schaffer-Hartmann copper reagent with the Folin-Wu phosphomolybdic acid; (*b*) Harding's copper reagent with the Nelson arsenomolybdic acid; (*c*) Somogyi's copper reagent with the Folin-Wu phosphomolybdic acid; and (*d*) Somogyi's copper reagent with the Nelson arsenomolybdic acid.

Much difficulty has occurred during the war years and subsequently in this country with colorimetric methods for blood sugar. Biochemists have repeatedly complained of the rapid fading of the blue colours. This fault has been more manifest because of the recent introduction of photo-electric instruments in which the rapid falling-off of the intensity of the blue colour has been noted by numerous workers. With Duboscq colorimeters the fading would not have been so readily noticed, since the rate of fading in standard and test is very similar. It has frequently been impossible during the last several years to obtain chemicals of Analytic Reagent quality, and this abnormal behaviour has been traced to the use of inferior chemical reagents. The ordinary or technical grade of sodium carbonate, for instance, contains considerable sodium bicarbonate as well as the sodium carbonate. The amount of this inferior reagent specified for the preparation of an alkaline copper solution contains insufficient sodium carbonate to produce the desired properties in the final solution, and an abnormal behaviour consequently results.

Experimental

Reagents

Harding's copper reagent.—The modified Harding-Schaffer-Hartmann reagent is described by Haslewood and Strookman (1939). This reagent is identical with that described by Harding and Downs (1933), except for the omission of the potassium iodide. Its preparation is also described by King (1947).

Somogyi's copper reagent.—Prepared according to Somogyi (1945).

Folin and Wu's phosphomolybdic acid.—Prepared according to Folin and Wu (1920).

Nelson's arsenomolybdic acid.—Prepared according to Nelson (1944), King (1947).

Glucose standards.—A stock solution is made by dissolving 0.1 g. anhydrous glucose in saturated benzoic acid solution and making up to 100 ml.; 2, 3, and 5 ml. of the stock solution are diluted to 100 ml. with saturated benzoic acid to give working standards of 0.02, 0.03, and 0.05 mg. per ml. respectively (equivalent to 80, 120, and 200 mg. glucose per 100 ml. of blood, according to the following method).

Isotonic sodium sulphate-copper sulphate solution.—3 g. Na_2SO_4, 10 H_2O, and 0.6 g. $CuSO_4$, 5 H_2O are dissolved in water and made up to 100 ml.

Sodium tungstate.—10 g. per 100 ml.

Procedure

Preparation of blood filtrate.—0.05 ml. of blood is added to 1.85 ml. of the isotonic sodium sulphate–copper sulphate solution in a conical centrifuge tube, and 0.1 ml. of sodium tungstate is added. The mixture is shaken, and the precipitated proteins and copper tungstate are spun down in the centrifuge, or filtered through a small paper.

Estimation of glucose.—1 ml. of the above filtrate and 1 ml. each of the glucose standard solutions are measured into 3/4-in. diameter test tubes; 1 ml. of copper reagent is added and mixed. The test tubes are stoppered with cotton-wool and heated in a boiling water bath for exactly 10 minutes. They are cooled in running water, and 3 ml. of Folin-Wu phosphomolybdic acid, or 1 ml. of Nelson's arsenomolybdic acid, with water to 5 ml. (for Duboscq; 10 ml. for photo-electric) are added. The colours are allowed to develop for 10 minutes and are then compared.

Results

Reagents Prepared from "Ordinary Grade" Chemicals

Comparison of colorimetric methods.—Several dilutions (in addition to the above) were made of the stock standard glucose solution. One ml. samples were treated according to the above procedure and the colours measured in a photo-electric absorptiometer with the Ilford spectrum red filter. The zero adjustment was made with water in the cuvette. The results (Fig. 1) showed that the combinations of Harding with Folin-Wu, Harding with Nelson, and Somogyi with Nelson, furnish procedures capable of giving good conformability with Beer's Law; but that the Harding–Folin-Wu mixture produced a considerably larger blank than the others. The Somogyi–Folin-Wu combination yielded much less satisfactory figures whose proportionality fell off rapidly with increasing concentrations of glucose.

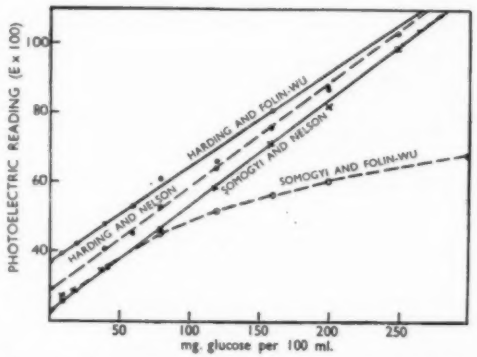

FIG. 1.—Photo-electric readings (E × 100) of glucose solutions estimated by different methods. Chemicals of ordinary quality (not analytical reagent grade) were used in preparing the reagents. Note the high "blank" colours.

Stability of colours produced.—Standard solutions containing amounts of glucose equivalent to 60 and 120 mg. glucose per 100 ml. and a water blank were prepared with the Harding copper and Folin-Wu phosphomolybdic acid. Rapid fading was found to occur in the final blue solutions with loss of proportionality (Fig. 2). It will be seen that a considerable amount of reduction occurred in the blank. This was, in fact, visible to the naked eye, there being a greenish precipitate of cuprous oxide when the tube was removed from the water bath.

The reagents used in the above experiments had been prepared when no Analytical Reagent chemicals were available. It had previously been pointed out from this laboratory (King, Haslewood, and Grant, 1936) that impure sodium carbonate cannot be used for the titrimetric method for glucose, and it is apparent now that it is equally dangerous to use it for the colorimetric procedures. It was observed that when the Folin-Wu phosphomolybdic acid was prepared from wartime molybdic acid, a considerable amount of ammonia was evolved when the sodium hydroxide was added in the preparation of the reagent.

Reagents Prepared from Analytical Reagent Chemicals

Fresh copper reagents were now prepared in which Analytical Reagent chemicals were used throughout. The sodium carbonate was Kahlbaum pro. anal. (Griffin and Tatlock); the other chemicals were from British Drug Houses.

Fresh phosphomolybdic acid was prepared using an equivalent amount of sodium molybdate (instead of molybdic acid and sodium hydroxide). 44.3 g. Na_2MoO_4,H_2O and 5 g. Na_2WO_4, $2H_2O$ were dissolved in 250 ml. distilled water containing 2.8 g. NaOH, and the solution boiled for 30 minutes. Water was added to approximately 350 ml., followed by 125 ml. H_3PO_4 (89 per cent w/v; sp. gr. 1.75) and the volume made up to 500 ml.

Blank colour.—The use of these reagents resulted in the elimination of the high blank

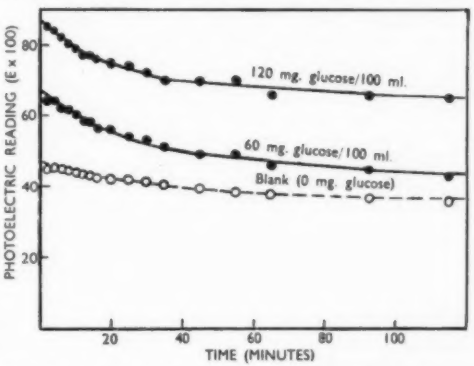

FIG. 2.—Photo-electric readings (E × 100) of glucose solutions estimated by the modified Harding copper reagent and the Folin and Wu phosphomolybdic acid reagent. Ordinary quality chemicals—not Analytical Reagent grade. Note the high "blank" readings and the rapid fading of the colours.

readings with the Harding with Folin-Wu combination (Haslewood and Strookman method). Blanks prepared from fresh copper reagents heated with water (instead of glucose solution) gave a deflection of approximately a division on the absorptiometer when the zero adjustment was made with distilled water.

Stability of colour.—Water blanks and standard solutions containing an amount of glucose equivalent to 80 mg. per 100 ml. were prepared, using fresh copper reagents, and the colours developed with either phosphomolybdic or arsenomolybdic acid. The colours obtained were compared in a Hilger Spekker absorptiometer, using an Ilford spectrum red 608 filter. The zero adjustment was made with the blank solutions. Results are shown in Table I. Except in the case of the Somogyi with Folin-Wu combination, the colours remained substantially stable over a three-hour period.

TABLE I

STABILITY OF COLOUR IN AN 80 MG. GLUCOSE STANDARD DURING THREE HOURS

(Figures represent photo-electric readings, E × 100)

Time (mins.)	Harding and Folin-Wu	Harding and Nelson	Somogyi and Folin-Wu	Somogyi and Nelson
2	22.5	24	23	24.5
10	22.5	25	22.5	25.2
20	22.5	25	21.5	26
30	22.5	25	20.5	26
60	22.2	25	19.5	26
180	22	25	17.5	25

The development of colour in a similar series, in which the colour development was followed for eighteen hours and the blank colours were read with a water adjustment of the instrument zero, is illustrated in Table II. In every case, and particularly in the blanks, the colour becomes more intense with the lapse of time; but the difference between the standard and the blank readings becomes less except in the case of the Harding with Nelson combination, where it becomes greater. Thus, although there is an actual intensification of colour in every case, there is, except with the Harding–Nelson, a "relative fading" (that is, a lessening of the difference between standard and blank readings), which is most marked in the Somogyi with Nelson combination.

Proportionality.—The relation between colour development and concentration of glucose with the fresh copper reagents is shown in Fig. 3. Beer's Law is closely adhered to, even in the low range of sugar values, particularly with the Harding–Nelson combination. The Harding–Folin-Wu and the Somogyi–Nelson combinations also give satisfactory results, but the Harding–Nelson combination is preferred because of the greater stability of its blank colours.

Discussion

The above results emphasize the necessity of preparing sugar reagents from the best chemicals obtainable. Only Analytical Reagent chemicals should be employed. The use of reagents prepared from impure chemicals can only lead to the production of unreliable results.

The purity of sodium carbonate seems to be the most important factor in the production of satisfactory copper reagents. If Analytical Reagent sodium carbonate cannot be obtained, the ordinary sodium carbonate should be heated in a crucible to red heat and maintained at that temperature for about thirty minutes. The cooled product is powdered in a mortar and stored in a bottle with a tightly fitting stopper. This treatment ensures that any contaminating sodium bicarbonate is decomposed into sodium carbonate. The procedure,

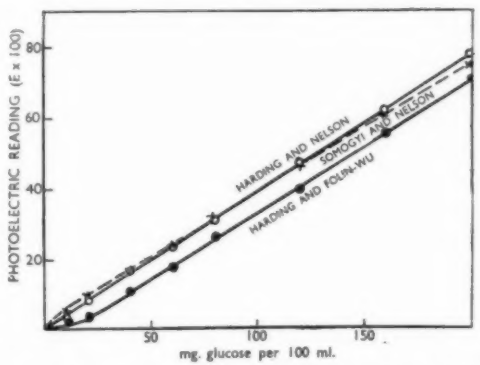

FIG. 3.—Calibration curves for glucose methods with reagents prepared from Analytical Reagent grade chemicals. Photo-electric readings (E × 100) with the Hilger Spekker absorptiometer, 1 cm. cells and Ilford spectrum red filters.

however, is only recommended when Analytical Reagent sodium carbonate is not available.

Some samples of Nelson arsenomolybdic acid, prepared strictly according to Nelson's instruc-

TABLE II

STABILITY OF COLOUR OF A 200 MG. GLUCOSE STANDARD DURING EIGHTEEN HOURS

(Figures represent photo-electric readings, E × 100)

Time	Harding and Folin-Wu			Harding and Nelson			Somogyi and Folin-Wu			Somogyi and Nelson		
	200 mg. std.	Blank	Diff.	200 mg. std.	Blank	Diff.	200 mg. std.	Blank	Diff.	200 mg. std.	Blank	Diff.
(min.) 5	63	3.5	59.5	77.5	14	63.5	76	18.5	58.5	80	19.5	60.5
(hr.) 1	64.5	5	59.5	78.5	14.5	64	77.5	22.5	55	83	23	60
18	72	21.5	50.5	127	18	109	78	40	38	107	85	22

tions, give a persistent green colour with either the Harding or Somogyi copper reagents. Other batches give a green colour which with Harding copper reagent develops into a blue colour within 5 minutes, while with the Somogyi reagent the colour retains a definite greenish tint for about one hour. It has also been found that the readings of blanks prepared from the Harding or Somogyi reagents increase slightly with the age of the reagent. Neither of these points affects the accuracy of the results when the readings of the colours are made with a red light filter, and when, with a photo-electric instrument, the zero setting is made with a blank solution.

Summary

1. The Harding copper reagent (without the iodate) proposed by Haslewood and Strookman gave better colour development both with Folin and Wu phosphomolybdic acid and with Nelson arsenomolybdic acid than did the corresponding Somogyi reagent.

2. The colour development obtained with Harding reagent is of the same order as that obtained with Somogyi reagent.

3. The Harding–Folin-Wu and Harding–Nelson combinations are found to give the more stable colours, the Somogyi–Nelson and the Somogyi–Folin-Wu combinations being subject to varying degrees of fading.

4. With low sugar concentrations the Harding–Nelson combination is found to give more reliable results than the Harding–Folin-Wu.

5. Only chemicals of Analytical Reagent (A.R.) quality should be used for the preparation of blood sugar reagents.

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J. clin. Path. (1947), **1**, 34.

FAMILIAL INTESTINAL POLYPOSIS

BY

CUTHBERT E. DUKES

From St. Mark's Hospital, London

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Familial polyposis is of interest for its own sake as a sort of pathological curiosity and also in relation to the problem of cancer. The term "polyposis" is used to describe a widespread adenomatous proliferation of the intestinal mucous membrane, leading to the formation of multiple adenomata. It should not be applied to intestinal polyps of different histological structure, such as inflammatory or fibroid polyps, because these exhibit no special tendency to carcinoma, whereas true polyposis undoubtedly does. In fact, intestinal cancer is an almost invariable sequel to familial polyposis.

Clinical Classification of Adenomatous Polyps

Two clinical types of multiple adenomatous polypi may be distinguished: (1) a *familial* variety which affects several members of one family and generally manifests itself in adolescence; and (2) a so-called *acquired* variety which usually appears later in life and is not hereditary. This classification of intestinal polypi was first suggested by Erdmann and Morris (1925) who pointed out that there are some features in which these two clinical conditions resemble each other and some in which they differ. Both types of polypi tend to malignancy, but in the familial or adolescent variety the polyps are very widely disseminated throughout the rectum and colon, whereas the acquired or adult type is more limited in numbers and extent. Also the liability to cancer is greater in the familial than in the acquired type.

Differentiation of the Familial Type

Although these points of distinction between the two varieties are of value, they do not always serve to settle at once whether a case of multiple polypi is of the familial or acquired type. In most cases this can only be decided by inquiring carefully into the family history. When this is done there is often ample evidence that near relatives have been similarly affected and the case is obviously of the familial type. In other instances there is nothing to suggest that any near relative has had any complaint which could be attributed to polyposis. Our experience at St. Mark's Hospital is that evidence of a familial character is obtained in about one-third to one-half of all polyposis patients at the time of first diagnosis, and this is in close agreement with the experience of McKenney (1936) and Friedell and Wakefield (1943). However, this is certainly an underestimate of the extent of the familial character, because if the distinction between the familial and the acquired type of polyposis is only to depend on the presence of polypi in other members of a family then obviously the first victim in a family will always be classified as "acquired." Only when subsequent cases have occurred among relatives will the familial character be apparent. For this reason cases provisionally classified as of the acquired type may need to be reconsidered if evidence of a familial disposition is discovered later.

Development of Knowledge of Familial Polyposis

Polyposis is a relatively rare disease, and before the closing years of the nineteenth century nothing was known of its familial character or liability to cancer, though a few descriptions of multiple polypi can be found in the medical literature of that period. Some of the reported cases were probably examples of inflammatory polyps following chronic ulcerative colitis or dysentery; in fact, it was generally supposed in those days that all polyps were the late result of an inflammatory process. The credit for recognizing the familial character of some forms of polyposis should be given to Harrison Cripps (1882), who sixty-five years ago read a paper before the Pathological Society of London entitled "Two cases of disseminated polypus of the rectum." The interest of this communication lay in the fact that the two patients, a boy of 19 and a girl of 17, were brother and sister. Within a few years several other examples of familial polyposis were reported, and the fact became established that heredity is an

important consideration in relation to some intestinal polypi.

Eight years later Handford (1890) also presented a paper before the same society in which he described a case of polyposis in a woman aged 34 who died from cancer of the rectum. Similar cases were soon recorded in other countries and evidence began to accumulate both of the familial character and of liability to cancer.

The introduction of the sigmoidoscope resulted in a rapid advance in knowledge of this disease; and Lockhart-Mummery, who was one of the first surgeons in England to advocate the more general use of the sigmoidoscope, was also one of the first to publish genealogical trees depicting the transmission of polyposis from one generation to another (Lockhart-Mummery, 1925). His paper illustrated the inheritance of polyposis in three families, and five years later further information about these families and other similar cases was recorded (Dukes, 1930). Since then many more cases have been reported.

Morbid Anatomy and Histology of Polyposis

In the familial type of polyposis the whole mucosal surface of the colon and rectum is covered with innumerable tumours ranging in size from tiny mammillations, only just visible, up to large pedunculated tumours. The smaller tumours are sessile (Fig. 1), but the larger ones are often attached by a broad strap-like stalk (Fig. 2). The total number of tumours varies considerably. They may cover the whole surface diffusely so that it is hard to find an unaffected patch of mucous membrane, or they may be more sparsely scattered; but there are always large numbers of tumours. The disease generally affects the whole colon and rectum but not the small intestine and stomach, though occasional isolated polyps may be found in the stomach also (Coffey and Bargen, 1939). As a rule, however, the disease ends abruptly in the ileo-caecal junction.

Sections show the tumours to be adenomas in which active epithelial proliferation is in progress. All stages in the development of an adenoma may be noticed, the commonest lesions being small adenomas with a short pedicle (Fig. 3). In between the tumours the intestinal mucous membrane may show patches of hyperplasia.

When examining a case of polyposis a special search must be made for signs of carcinoma. The features which suggest malignancy are darker colour, firmer consistency, and ulceration. The adenomata themselves are often darker in colour than the surrounding mucosa, but a focus of carcinoma makes such a tumour even darker still. Most adenomata feel soft and have a rubber-like consistency, but when malignancy has supervened they feel much harder and more solid. Ulceration is almost invariably a sign of malignancy, and so is induration of the stalk of a pedunculated tumour.

Symptoms and Diagnosis of Polyposis

In familial polyposis the onset of symptoms is usually insidious and there is at first only slight looseness of the bowels associated with the passage of small quantities of blood and mucus. At first the patient is not ill in the clinical sense. This is a point of distinction between polyposis and polypoid lesions of an inflammatory nature following colitis or dysentery, because in these the initial illness is of a severe character. On the other hand, with familial polyposis the initial symptoms are relatively trivial. For instance, in two cases recently encountered the patients had suffered from symptoms of polyposis for seven years before consulting a doctor.

The final diagnosis of polyposis generally depends on the result of sigmoidoscopic examination, but radiographs are sometimes a valuable aid to diagnosis. Anderson and Marxer (1930) have pointed out that in polyposis the mucosa in general presents a mottled or honeycombed appearance, but frequent examination may be needed to demonstrate this well, and the general routine of the ordinary barium meal may require some modification.

Inheritance of Polyposis

It is well known that, although cancer itself is not inherited in the ordinary sense of the word, none the less certain families do show a strong predisposition to the development of tumours. In these families tumours of a specific type tend to appear in a specific organ (Macklin, 1935). The simple occurrence of two or more cases of cancer in one family does not of course prove that any inherited predisposition exists. The operation of the law of chance will naturally result in some families having an excess of cancer and others none at all. But, as Weller (1937) has pointed out, there is ample evidence of the existence of some families in which the mass incidence of cancer is significantly in excess of all normal expectations, and he quotes as an example the family originally studied and reported by Warthin. Forty-one out of 174 members of this family attaining the age of

25 years developed neoplasms. With two exceptions all the cancers were of the gastro-intestinal tract and uterus. No less than 20 of the males had suffered from cancer of the intestinal tract.

Members of polyposis families show no special proclivity to the development of tumours in any other organ of the body than the intestine, and the disease does not usually manifest itself before childhood or puberty although the age at which adenomata develop varies considerably in different families. For the cases we have investigated at St. Mark's Hospital, London, the average age at which adenomata were discovered was 22 years. The youngest patient was 8 and the oldest 45. There was reason to believe that the tumours had been present for a year or two before they were discovered, so they probably developed at about 20 years of age on the average. Gabriel (1945) states that only rarely has polyposis been noticed in the first decade or after forty years of age. Occasionally, however, cases have been reported which suggest that polypi have been present at birth or developed in infancy. Thus McKenney (1936 and 1939) reports one family in which he sigmoidoscoped four children aged 2, 5, 9, and 11, and found adenomas in each. These tumours seemed to be progressively larger and more numerous as age advanced, and he considered that in these four children polypi had probably been present at birth.

One of the unsolved problems in the pathology of polyposis is whether all or only some members of a family inherit the mysterious defect which manifests itself finally as polyposis. In a decent-sized, intelligent, and co-operative polyposis family one can generally get evidence that about half the adult members are or have been affected. The question is whether the at present unaffected individuals are destined to develop the disease later. The only way to answer this conundrum is to watch to see what happens to them. At St. Mark's Hospital we have engaged in this waiting game for more than 20 years and have had the opportunity more than once to observe polypi develop in an individual who at the first sigmoidoscopic examination was certified free from polyps.

A striking example of this is seen in the genealogical tree recorded in Fig. 4. In this chart males are represented by squares, females by circles, and individuals with intestinal tumours (whether adenomas or carcinomas) by black squares or circles respectively. The man recorded as III 1 (indicated by an arrow) is now 60 years of age. His father, grandfather, and at least five of his uncles and aunts died of cancer, and some of them had transmitted polyposis to their descendants. This patient was first examined by Mr. Lockhart-Mummery at the age of 38 and was found to be free from polypi. He was kept under observation, and six years later, when he was 44, polypi were first noticed. The patient was quite well aware of the existence of the skeleton in the family cupboard and he kept closely in touch with his medical advisers, which proved to be a good policy for him, because at the age of 56 he developed cancer of the descending colon. This was successfully excised by Mr. Lockhart-Mummery, but three years later at the age of 59 he developed cancer of the rectum, which was dealt with by Mr. Naunton Morgan by perineo-abdominal excision. A few months later, at the age of 60, the patient developed a third cancer at the site of his colostomy, and this was also removed without delay.

This patient's story illustrates the difficulty of deciding whether the defect leading to polyposis is inherited by all or only by some members of a family. On the first occasion when the pedigree of this patient was published (Lockhart-Mummery, 1925) he was represented as unaffected. When further details were published by Lockhart-Mummery and Dukes in 1939 he was recorded as suffering from polyposis. Now the story has unfolded itself still further, and he is recorded as an example of multiple cancer.

This incident also illustrates the fact that a polyposis family group is constantly changing its composition and the proportion of unaffected individuals varies from time to time. McKenney (1939), who has kept careful records of three families for several years, has described tersely the vigilant attitude which must be adopted by the student of the disease. "A polyposis family group history is an ever-changing one," he says, "and the chronicler must not lay aside his typewriter as the story must constantly be rewritten."

There are two ways in which further knowledge may be gained about the transmission of polyposis. One is by patiently observing year by year and recording what happens in polyposis families. The other is by searching for curious and unexpected incidents which may throw light on the problem.

There is, for instance, the family reported by Zahlman in which a woman free from polyposis was married twice (for reference see Dukes, 1930). By her first husband, who was also free from polyposis, she had two children, both of whom were healthy. Her second husband suffered from polyposis, and ultimately died of cancer. By him she had four children, all of whom suffered from polyposis. This proves transmission by the male.

Again, in a family recorded by McKenney (1936), a woman suffering from polyposis was married twice, each of her husbands being free from the disease.

She had four children, two by each husband, and all four children suffered from polyposis. This proves transmission by the female.

One of the families studied at St. Mark's Hospital is of special interest because of the presence of twins in the second generation. Each of the twins developed polypi about the age of 30. One was treated by colectomy at the age of 41 and succumbed to the operation: the other also died of intestinal cancer at the age of 41.

Polyposis patients sometimes ask the question: Who was responsible for first introducing this defect into our family and what is it due to? This, of course, is another unsolved problem, but a clue to it might be obtained if family pedigrees could be traced further back. People generally know something about the health of their parents, but very little about their grandparents and practically nothing about their great-grandparents, so it is seldom possible to trace a complaint through a family for more than two or three generations.

When further data are available it may possibly be found that polyposis results from the chance mating of two individuals, each of whom was destined to die of cancer of the intestine. At any rate it is worth recording that in two of the ten families we have investigated at St. Mark's Hospital the disease appeared in a family in which both father and mother had died of cancer of the rectum or colon. As far as could be ascertained this was the starting point of the disease in these two families.

Relationship of Polyposis to Carcinoma

Malignant disease secondary to familial polyposis is characterized by its early age of onset and the fact that more than one primary focus of carcinoma may be present. Among the general population it is rare for cancer of the rectum or colon to develop before the age of 40, but in families affected by polyposis it develops in the thirties or even earlier. The age at death from intestinal cancer in polyposis families is younger by about twenty years than the average age of death from intestinal cancer in the general population. There is considerable variation in different families, but in most cases it may be said that polypi may be expected before the age of 20 and cancer is liable to manifest itself some ten to fifteen years later.

Polyposis is not a familial disease which is likely to spread and become more prevalent: on the contrary polyposis families tend to die out. That is because cancer so frequently develops before the age of marriage and because even those who do marry die young. Moreover there seems to be a tendency for malignant disease to begin at an earlier age-period in each succeeding generation.

The treatment of polyposis presents a difficult problem. Constant medical supervision is necessary so that a radical excision may be undertaken as soon as malignancy is detected. No other method of treatment than surgery has any permanent effect on the course of the disease.

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FIG. 1.—Surface view of mucous membrane of colon in familial polyposis, showing sessile adenomata.

FIG. 2.—Pedunculated adenomata in familial intestinal polyposis.

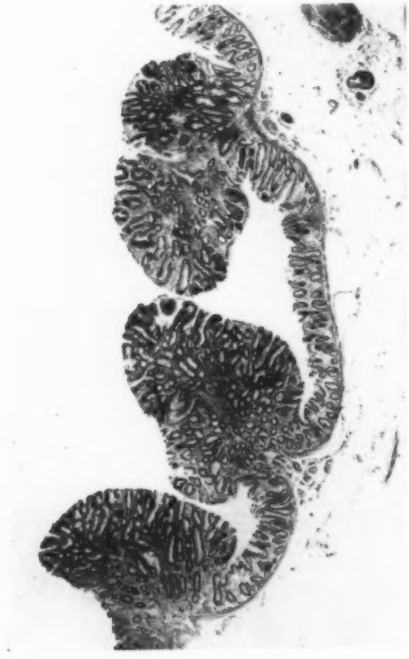

FIG. 3.—Section through rectal mucosa in a case of adenomatosis (×8). Reproduced by kind permission of the *Lancet*, from the article by Lockhart-Mummery and Dukes (1939).

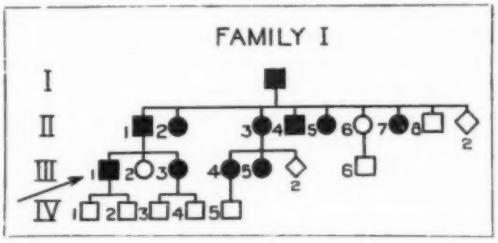

FIG. 4.—Family tree, showing: I Death from cancer of rectum, aged 42; II (1, 2, 3, 4, 5, 7) Death from cancer of rectum, aged 42, 52, 54, 39, 27, and 44 years respectively; III (1) Polypi at age 44, carcinoma of splenic flexure at 56, carcinoma of rectum at 59, and carcinoma in colostomy at 60 years; (3) Polypi at age 37; (4) Polypi at age 11: colectomy for polyposis at 31: death from cancer of rectum at 52; (5) Death from cancer of colon aged 33 years.

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REPORT ON TWO SPECIES OF THE GENUS FUSIFORMIS OBTAINED FROM PATHOLOGICAL LESIONS IN MAN

BY

HERTA SCHWABACHER AND D. A. MITCHISON

E.M.S. Sector IV Central Laboratory, Watford, Herts

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It is at present very difficult to classify accurately a given strain of the genus *Fusiformis*, and the following case histories may therefore be of interest.

Clinical Histories

Strain 1.—A woman aged 46 years was admitted to Shrodells Hospital, Watford, Hertfordshire, under the care of Dr. Duffus on Jan. 12, 1945, complaining of severe abdominal pain. Since Dec. 25, 1944, she had noticed frequency and pain on micturition. Her periods had been normal and bowels regular. Abdominal pain had become severe on Jan. 8. Her own doctor had administered 15 g. sulphonamide; chemotherapy was discontinued in hospital.

On admission, her temperature was 98° F.; pulse 112. There was extreme tenderness in the left inguinal region. Vaginal examination revealed fullness in the left fornix. The urine was normal. The blood showed leucocytosis and a "shift to the left" (red blood cells 5,090,000 per 100 c.mm. of blood; Hb 70 per cent; colour index 0.7; white blood cells 17,200; polymorphs 75 per cent, band forms 15 per cent; eosinophils 2 per cent; basophils 1 per cent; lymphocytes 18 per cent; large mononuclears 4 per cent.

A laparotomy was performed by Dr. Duffus on Jan. 12, 1945. One pocket of excessively foetid pus was found in the pouch of Douglas, and another pocket on the outer surface of the descending colon at the level of the iliac crest. The left Fallopian tube was dilated and inflamed. There was no diverticulitis or other abnormality of the abdominal viscera. A drainage tube was left in situ and the abdomen closed. There was free drainage of pus for several days. The patient made an uneventful recovery and was ready for discharge on Feb. 2, 1945.

Bacteriological examination of pus from the pelvic abscess.—Direct examination showed degenerate pus cells, numerous Gram-negative filamentous bacilli, and a few Gram-positive cocci in chains. The material was inoculated on to two horse-blood agar plates containing 6 per cent agar (Hayward and Miles, 1943), and on Nagler plates (Hayward, 1943), and into a tube of Robertson's cooked-meat medium. The Nagler plate failed to show the presence of *Cl. Welchii* or *Cl. bifermentans*. One blood-agar plate incubated aerobically remained sterile; the second blood-agar plate, incubated anaerobically, when examined after seventy-two hours showed (1) numerous colonies, 0.2 to 0.5 mm., opaque and slightly haemolytic, which were found to be composed of Gram-negative pleomorphic cells; (2) seven-minute "dewdrop" colonies (in the pool), which finally conformed to Prevot's classification of *Str. micros.* The cooked-meat medium yielded *Ps. pyocyanea* in addition to these organisms.

Because of the resemblance of the anaerobic Gram-negative bacilli to those seen in profusion in direct smear, they were considered to be the cause of the abscess.

Strain 2.—A woman aged 62 years was admitted to Shrodells Hospital under the care of Dr. Roberts on July 4, 1945; she complained of a painless swelling in the region of the pubic symphysis which she had had for 3 or 4 days before admission. During this time she had also had diarrhoea. Her temperature was 102.6° F. The lump in her pubic region was situated superficially, was 3 inches in its largest diameter, and was surrounded by a zone of erythema. She was placed on full dosage of sulphathiazole and her temperature rapidly fell to normal. On July 6 the abscess was opened and a large gangrenous slough removed. At the same time pus was taken for culture. The abscess healed well, and on Aug. 7 was only discharging slightly. On July 30 a radiograph of the pelvis failed to reveal any bony abnormality.

Bacteriological examination of the pus.—Direct examination showed pus cells, numerous Gram-negative bacilli, and a few Gram-positive cocci. The material was inoculated on to two horse-blood agar plates, containing 2 per cent agar, one of which was incubated aerobically and the other anaerobically. On the aerobic plate there was a scanty growth of three types of colony. One of these was a beta-haemolytic streptococcus which did not belong to Lancefield's

groups A, B, C, or G. The other two were diphtheroids.

On the anaerobic plate, after forty-eight hours' incubation at 37° C., there was a heavy growth of colonies about 0.6 mm. in diameter, consisting of small Gram-negative bacilli. In view of its preponderance and the fact that the streptococcus did not belong to a Lancefield's group that is normally pathogenic in man, this anaerobic organism was probably the principal pathogen. As regards the remaining organisms isolated from the pus, the Gram-negative anaerobic bacillus was later found to demonstrate satellitism to one of the diphtheroids obtained from the aerobic plate.

Description of the Two Strains of Gram-negative Anaerobic Bacilli

Morphology.—The bacilli were grown on a Fildes slope and incubated in an atmosphere of 5 per cent CO_2 and hydrogen for seventy-two hours.

Strain 1.—The cells varied considerably in length, being 0.5×1.0–10.0 μ. They were Gram-negative bacilli and showed irregular staining, and globoid or oval bodies, 1.3 μ in diameter, lying free or along the bacilli, swollen-ended bacilli, or filaments having a central distension. Many long filaments were curled upon themselves. Some were branched, but it was difficult to tell whether this was true or false branching. Cocco-bacillary forms

Strain 1, showing filamentous forms and globoid bodies when grown on enriched media. (×1,000.)

occurred in unenriched media. The organisms were non-motile, this being confirmed by flagella staining; non-capsulated; and did not contain spores (a culture did not survive heating for ten minutes at 80° C.). The organism was not acid-fast.

Strain 2.—The organism was a small Gram-negative bacillus, with parallel sides and rounded ends, about 0.2×0.6–2.5 μ. It stained uniformly with carbol fuchsin, showing little pleomorphism and no branching. No capsules or spores were demonstrated, and it was not acid-fast. In Fildes broth incubated under the same conditions there was rather more pleomorphism with coccal and long filamentous forms up to 10 μ in length. Large globoid bodies or distensions in the filaments were never seen. The organisms were non-motile, as confirmed by flagella staining.

Colonial morphology.—Colonies were grown on blood-agar plates containing 2 per cent agar and incubated at 37° C. for seventy-two hours in a microaerophilic atmosphere (i.e., the pressure in a jar was lowered to 260 mm. Hg and the vacuum replaced with hydrogen.

Strain 1.—The colony was between 1 and 1.5 mm. in diameter, grey, circular, with an entire edge, semi-translucent, slightly haemolytic, but never frankly beta-haemolytic, raised, convex, smooth, shiny, compact, of a butyrous consistency, and easily emulsifiable.

Strain 2.—This was similar to strain 1, but no haemolysis was present.

Growth requirements

Strain 1.—After recent isolation, the strain failed to grow in peptone water, broth, or gelatine agar. The addition of Fildes extract in a concentration of 1 per cent yielded prolific growth. Peptone water with V factor, with X factor, and with V and X factors yielded growth. Satellitism to a *Staph. albus* colony on an agar plate was marked after seventy-two hours' incubation. About six months after isolation the organism was found to be capable of growing in peptone water and broth.

Cultures incubated in 5 per cent carbon dioxide in hydrogen, under microaerophilic conditions and under anaerobic conditions yielded colonies whose mean diameters were 0.60 mm., 0.44 mm., and 0.08 mm. respectively. No growth was obtained in 5 per cent carbon dioxide in air, or aerobically. Fildes-agar shake cultures showed a heavy band of growth 12 mm. below the surface of the agar. Smaller colonies grew right to the bottom of the tube, but no colonies developed above the band. For up to five days the colonies were translucent, lenticular, and gave the appearance of gas bubbles; later they became opaque and the agar was split by gas.

Strain 2.—This was similar to strain 1, except that poor growth was obtained in peptone water soon after isolation. Addition of Fildes extract or V and X factors increased the amount of growth. In the Fildes-agar shake culture, growth was maxi-

TABLE I

	Strain 1	Strain 2	*F. necrophorus*	*F. fragilis*
Indole production	−ve	−ve	+ve	variable, usually −ve
Lactose fermentation ..	+ve	+ve	−ve	+ve
Glucose ,, ..	+ve	+ve	+ve	+ve
Sucrose ,, ..	+ve	+ve	variable, usually −ve	+ve
Maltose ,, ..	+ve	+ve	+ve	+ve
Mannitol ,, ..	−ve	−ve	variable, usually −ve	−ve
Fructose ,, ..	+ve[1]	+ve[1]	+ve	+ve
Glycerol ,, ..	−ve	−ve	variable	
Gas production in carbohydrate media	little	little	much	little
H_2S production[2]	+ve	+ve	+ve	−ve
Nitrate reduction	−ve	−ve	−ve	−ve
Litmus milk	Acid and clot after 7 days		No change	Some strains; acid and clot
Gelatin liquefaction	−ve	−ve	−ve	−ve
Liquefaction of inspissated serum	−ve	−ve	−ve	−ve
Catalase production	+ve	+ve		

[1] Acid but no gas in fructose.
[2] Growth on a Fildes slope tested by blackening of a lead acetate paper; +ve 35 mm. in 24 hours.

TABLE II

Date	Animal	Mode of inoculation	Killed	Enlarged glands		Organism recovered on culture from				Titre
				Inguinal	Sublumbar	Local lesion	Gland	Heart blood	Spleen	
*7.7.45 20.3.45	Mouse	Intramuscular aa 5% $CaCl_2$ and cooked-meat culture	9.7.45 26.3.45	+ +	+ +	+ +	− +	− −	− −	
20.3.45	Mouse	Intramuscular 0.5 ml. cooked-meat culture	26.3.45	+	+	−	−	−	−	
*31.7.45 20.3.45	Mouse	Intraperitoneal 0.2 ml. cooked-meat culture	10.8.45 26.3.45	− −	+ +	− −	− −	− −	− −	
26.3.45	Mouse	Intramuscular aa 5% $CaCl_2$ and cooked-meat culture	14.4.45	+	+	+	+	−	−	
20.3.45	Guinea-pig	Intramuscular 1.0 ml. cooked-meat culture	14.4.45	−	−	−	−	−	−	20
26.3.45	Rabbit	Intralabially. Left lip, 0.2 ml. $CaCl_2$ + 0.2 ml. saline Right lip, 0.2 ml. $CaCl_2$ + 0.2 ml. culture	30.5.45	−	−	−	−	−	−	< 2
26.3.45	Rabbit	Intravenous 1.0 ml. cooked-meat culture	30.5.45	−	−	−	−	−	−	16
	10 normal human sera									<20

* = Strain 2.

mal at 7 mm. below the surface, but no gas was produced. Colony size was increased in an atmosphere containing carbon dioxide as much as with strain 1.

In Fildes broth both strains produced an even turbidity with a slight ropy deposit after forty-eight hours.

Strain 1 survived three weeks in cooked-meat medium.

Heat resistance.—Both strains failed to survive five minutes at 60° C.

Biochemical activities.—These were tested with strips of iron in sugar tubes and the results are listed in Table I.

Drug sensitivity.—Both strains were sensitive to 5 mg. per cent sulphadiazine, sulphamezathine, sulphapyridine, sulphathiazole, and sulphanilamide (Harper and Cawston, 1945). The minimal bactericidal concentration of penicillin for strain 1 was 62 units per ml., while for strain 2 it was between 10 and 20 units per ml.

Biological tests.—From the biological tests of strain 1, listed in Table II, it will be seen that the strain proved completely non-pathogenic to the laboratory animals used. Mice receiving intramuscular inoculations of 0.25 ml. of 5 per cent calcium chloride with 0.25 ml. cooked-meat culture showed enlargement of their proximal glands. From these glands the fusiform organism was recovered on culture. Because of the morphological resemblance of this bacillus to *F. necrophorus*, a rabbit received intralabial inoculation. No necrosis developed during a two months' period of observation.

Serological examination of rabbits and a guinea-pig failed to reveal any agglutinin response to the organism inoculated ; similarly ten sera, sent to the laboratory for Wassermann test, failed to agglutinate a suspension of the test strain in a final dilution of 1 in 20.

In the case of strain 2, only mice were tested. They received intramuscular injection of the culture with calcium chloride and intraperitoneal injection with cooked-meat culture.

Discussion

Organisms of the genus *Fusiformis* are the predominant flora of the gastro-intestinal tract, and from faeces a very large variety of strains can be obtained with widely variable characteristics (Eggerth and Gagnon, 1933). Other members of the group cause suppurative lesions of mucous membrane and abscesses in tissues close to the gut, the vagina, and the genito-urinary system. They have been studied for many years from the bacteriological and clinical standpoint by French workers (Lemierre, 1936, who gives the main French references) and more recently in America (Dack, 1940, who gives a fairly full bibliography). In all probability infections with these organisms are more common than is realized in this country.

Of the various described species the following two have been studied most completely and are most closely related to the strains we have isolated. However, lack of uniformity in technique, inadequate description of individual strains, and conclusions based on the study of only a few strains make comparisons of different workers' results difficult. The first species, name *Fusiformis necrophorus* (Topley and Wilson, 1936), Schmorl's bacillus (Weinberg and others, 1937), *Bacteroides necrophorus*, etc., is probably identical with *Bacillus funduliformis* (Weinberg and others ; Dack and others, 1938). Fairly full descriptions have been given by Henthorne and others, 1936 ; Tessier and others, 1931 ; and by Dack and others, 1938. All authors agree that, morphologically, this is a non-motile, Gram-negative bacillus, usually ovoid, with biopular staining in exudates, and, in culture, highly pleomorphic, with long filamentous forms, often swollen. There are often large globoid bodies, irregular staining of the filamentous forms, and frequently false branching. The significance of the large spheroidal bodies found in cultures of "*Bacteroides funduliformis*" is discussed by Dienes and Smith (1944). They interpret them as a sign of a reproductive process different from binary fission. However, throughout a series of papers on the subject they do not appear to have investigated their strains apart from the morphology and growth requirements. In the full descriptions by the other authors quoted there is agreement that colonies are constantly haemolytic. Tessier and others describe haemolysis in blood glucose agar shake cultures resembling that of a beta-haemolytic streptococcus, while Dack and others state that on first removing a blood-agar plate from an anaerobic jar there is no haemolysis. As soon, however, as the blood becomes oxidized there is a wide area of green discoloration round the colonies, which usually changes to clear haemolysis on standing.

It appears that in liquid media there is a growth at the bottom of the tube and a clear supernatant fluid.

As regards biochemical activities, Table I illustrates the results obtained by these authors. No strains investigated fermented lactose. Some strains appear to be able to produce gas and a little acid in carbohydrate-free basic media, which may account for some of the discrepancies. All strains produce gas as well as acid with fermentable carbohydrates.

The organism is often pathogenic to rabbits, causing, on subcutaneous injection, an abscess which may spread and finally kill the animal with metastatic abscesses in the lungs. Intravenous injections may cause abscesses in the lungs, liver, and joints. Reports differ as to the pathogenicity of all strains. Those obtained from liver abscesses in cattle are more pathogenic than those from lesions in man. The mouse is less susceptible than the rabbit, while the guinea-pig is immune.

The second species is called *Fusiformis fragilis* (Topley and Wilson, 1936). It has been described by Henthorne and others (1936), and by Cohen (1932). In contradistinction to the first group, the organism is not pleomorphic and resembles a coliform bacillus in its morphology. The large spheroidal forms characteristic of necrophorus are not found. The colonies are usually smaller than those of necrophorus and are not haemolytic. In broth there is uniform turbidity. The biochemical reactions are given in Table I. In rabbits subcutaneous injection causes a local abscess, but in general the organism is less pathogenic than necrophorus.

The main distinction between necrophorus and fragilis is usually based on morphology, haemolysis, and the production of indole and hydrogen sulphide, but it appears to us that the fermentation of lactose is also important. However, in view of the fact that the pleomorphism of necrophorus is often present on certain media only, we feel that morphology is an uncertain criterion for differentiation.

As regards the two organisms described by us, No. 1 is similar to fragilis in all points except that of hydrogen sulphide production and of its morphology, which is typical of necrophorus. Methods of testing hydrogen sulphide production used in the reported descriptions of fragilis and necrophorus vary considerably in sensitivity, so that we doubt the significance of this difference. Furthermore, this organism would have had a morphology typical of fragilis had it been investigated in unenriched media alone, so that we are led to doubt the significance of a distinction between necrophorus and fragilis based on morphology alone. The haemolysis described for necrophorus is far more intense and of a character different from that observed in strain 1.

Strain 2 fits with the description of fragilis given above, again with the exception of hydrogen sulphide production.

Of the remaining members of the genus *Fusiformis*, *F. fusiformis* (Topley and Wilson, 1936) is separated off on the basis of having pointed ends, and *F. serpens* on motility and liquefaction of gelatin. There are other strains incompletely described.

In conclusion we must emphasize that the full importance of infections caused by these organisms will not be recognized until methods of anaerobic culture are used on a much wider scale than before. Few people realize that anaerobic technique is little more difficult than careful work with aerobic bacteria.

Summary

Two bacterial strains isolated from pathological lesions in man and belonging to the fusiform genus have been described, and their position in regard to the classification of this group has been discussed. They are most closely allied to the species *F. fragilis*.

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DIETHYLENE GLYCOL POISONING: REPORT ON TWO CASES

BY

ERIC WORDLEY

From the Pathological Departments, Prince of Wales's Hospital and City Hospital, Plymouth

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Few cases, if any, of death from drinking diethylene glycol have been reported in this country. This substance is, of course, largely used as an anti-freeze fluid in radiators of motor-cars, and is sold under a variety of trade names in this country, in America, and probably elsewhere, without any restrictions. There is little or no mention of the toxicity of this substance in British literature. It is not referred to in the latest editions of either Sidney Smith's (1943) or Glaister's (1945) textbooks, nor in Lucas's (1945) *Forensic Chemistry*. There is no mention of this substance in Hunter's (1943) monograph on "Industrial Toxicology." Bamford (1940) refers to the American "elixir" disaster referred to below. Brekke (1930) reported two fatal cases following the drinking of anti-freeze mixture.

An editorial article in the *Journal of the American Medical Association* (1937) describes and investigates a number of deaths in persons taking an "elixir of sulphanilamide." In all, seventy-three persons died as a result of taking this substance. The elixir was a mixture of 9–10 g. of sulphanilamide dissolved in 100 ml. of diethylene glycol. The dose recommended was three teaspoonfuls every four hours for one or two days. The most obvious symptom was anuria. Necropsy revealed a purplish mottling of the kidneys with necrosis in severe cases. It has been suggested by the American investigators of this disaster that sulphanilamide and diethylene glycol may produce additive toxic actions when given in combination.

Boemke (1943) reported an incident in which anti-freeze mixture was substituted for water in making coffee in a German military unit. Many men were dangerously ill and one died. It was suggested that death was due to uraemia caused by the oxidation of ethylene glycol to oxalic acid, which is deposited in the kidneys.

Pons and Custer (1946) describe details concerning ten soldiers who drank anti-freeze solution of ethylene glycol (Prestone type). Apparently this substance was drunk as a substitute for alcohol. They suggest that the minimum lethal dose is about 100 ml. The subjects were all young males. Death occurred in from twenty-two to forty-four hours after ingestion of the fluid. There was little opportunity to study clinical manifestations as all these cases were discovered in deep coma. Post-mortem findings showed generalized congestion of all organs and marked pulmonary oedema. The kidneys were swollen and displayed prominently engorged vessels. Crystals of calcium oxalate were prominent in microscopical preparations of the kidneys, mainly in the tubules. The brains were engorged and showed what the authors describe as "well developed encephalitis." They found numerous oxalate crystals in and about the engorged vessels of the brain. They suggest that coma and subsequent death were due largely to the cerebral complication. For this reason they also suggest that Brekke's two cases (1930) survived, not—as the author suggests—because there was unilateral decapsulation of the kidney, but rather because the amount of ethylene glycol they took was sublethal.

Milles (1946) reported a case of a man aged 30 who drank from a car radiator about 500 ml. of fluid that had had anti-freeze mixture added. The man died ten hours later with symptoms of heart failure and oedema of the lungs. At necropsy the kidney tubules were found to be swollen and disorganized. There were abundant deposits of calcium oxalate crystals in the tubules. He suggests, also, that the cause of death in ethylene glycol poisoning is essentially poisoning from oxalic acid, and cases should be treated as if that substance were the causal agent.

Accounts of the toxicology of the glycols are conflicting and not very precise. Page (1927) says he drank 15 ml. of ethylene glycol without ill effect.

Keston and others (1937) studied the toxic doses of diethylene glycol on rats and rabbits. A dose of from 1 to 2 ml. per kg. of diethylene glycol in rats was required to produce pathological changes, which consisted of extensive injury to the epithelium of the renal tubules, leading to urinary obstruction and uraemia. Holck (1937), in experiments on rats, found that 20 per cent of commercial diethylene glycol in their water killed all rats in about two weeks, and even 10 and 15 per cent proved fatal to some rats. Laug and others (1939) stated that ethylene glycol caused congestion and haemorrhage of the lungs. Smyth and others (1941) used rats and guinea-pigs. The animals were given the very large dose of 50 g. per kilo of various glycols by stomach tube. Most animals died within two days. The lethal dose of diethylene glycol appeared to be about 20 g. per kilo for rats.

Morris and others (1942) fed rats with ethylene and diethylene glycol at levels of between 2 and 3 per cent. The outstanding lesions were large stones in the bladder, tubular atrophy, and renal oxalate concretions. Most of the animals survived a two-year period of feeding. Sollmann (1942) described ethylene glycol as being twice as toxic as propylene glycol and half as toxic as diethylene glycol. Werner and others (1943) subjected rats to repeated exposures of vapours of ethylene glycol and related substances. The concentrations used were not enough to produce any obvious degeneration of the kidneys. Lehmann and Flury (1943) state that the effects of glycol poisoning are due to the formation of oxalic acid in the kidneys.

Case Reports

Case 1.—The fatal case now described was a young man aged 25, a German prisoner-of-war. He appeared back in camp with a bottle of fluid, saying that it had been given him as a present by a civilian. At the inquest later no light was thrown on how the man obtained this fluid. One evening he drank a good deal, perhaps the best part of half a winebottle, and offered some to others ; in fact two other prisoners had a little.

He was admitted to the City Hospital, Plymouth, and died about twenty-four hours after drinking the fluid. It was found later that the fluid in the bottle was pure diethylene glycol. On admission he was in a deep coma and deeply cyanosed.

Necropsy.—The body was that of a well-nourished very muscular young man. The face was deeply cyanosed and blood-stained, with froth oozing from the mouth and nose. The heart muscle was softer than to be expected in a healthy young man. The lungs were intensely congested and oedematous. The mucous membrane of trachea and bronchi were bright red in colour and full of blood-stained mucus. The liver was pale and fatty in appearance. The stomach and intestines showed no lesions. The kidneys were deep red and of a greasy appearance, and showed great engorgement of cortical vessels. The brain was very congested but showed no other abnormalities to the naked eye. The bladder was distended. The urine contained a cloud of albumin, and a few leucocytes and red cells were present.

Microscopically all the liver cells showed cloudy swelling, and there was a slight amount of fatty degeneration. The kidneys showed intense tubular degeneration and swelling ; hardly any nuclei were visible. In the lumen of numerous tubules crystals were prominent.

At first sight the appearances were those that would be expected in a case of poisoning by methyl alcohol, but by the time the necropsy was performed it was known that he had in fact drunk diethylene glycol, and particular attention was paid to the residual urine in the bladder and to the condition of the kidneys. At this time there had been no opportunity of surveying the literature, and it is regretted that a detailed microscopical examination was not made of the brain in view of Pons and Custer's observations referred to above.

Case 2.—The second man who also drank some of the fluid, perhaps 2 or 3 mouthfuls, was a healthy young man of 28 ; he had been slightly sick during the night following. He also was admitted to the City Hospital, Plymouth. On admission his colour was good but the tongue was furred ; he had slight headache and some vomiting shortly after admission. He passed 27 oz. of urine during the night. The next day he felt better and was allowed up. On the sixth day after admission, however, he had repeated fits, his blood urea was found to be 390 mg. per 100 ml., and there was almost complete anuria ; now that the cause of death, the nature of the fluid drunk, and the post-mortem findings of the first case were known, it was decided to perform decapsulation of the kidneys. This was done by the Medical Superintendent of the City Hospital, Mr. G. Larks.

Operation.—At operation the capsule was stripped from each kidney. There was considerable peri-renal oedema. Each organ was enlarged, congested, and bluer than normal. The tension inside the capsule was such that on incision of the capsule the kidney bulged quite markedly, the capsule stripped without

assistance, and the surface of the kidney became immediately redder.

The anaesthetic employed was a "high spinal," using novocain with pentothal, nitrous oxide, and oxygen.

Progress.—The man steadily improved and made an uneventful and perfect recovery. The blood urea findings were:

Just before operation, 390 mg. per 100 ml.
2 days after operation, 310 mg. per 100 ml.
3 days after operation, 210 mg. per 100 ml.
5 days after operation, 105 mg. per 100 ml.
10 days after operation, 30 mg. per 100 ml.

Case 3.—The third case was also a healthy young man, who is said to have had just a taste of the fluid. On admission to the City Hospital his condition was described as good, and he was in fact admitted only as a precaution. He had a little vomiting and headache during the night following the drinking of the fluid. There was a considerable amount of albumin in the urine, but no cells or casts. His blood urea on admission was 90 mg. per 100 ml. He made an uninterrupted recovery.

Comment

Without doubt diethylene glycol is a very toxic substance; if any general tendency develops to drink it as a substitute for alcohol, the results may be disastrous. The range of the lethal dose in man seems to be quite unknown.

Methods of treatment require investigation, and it has still to be decided whether or not treatment should be that for oxalic acid poisoning; it seems certain that the kidneys bear the brunt of the damage.

It would seem from the second case quoted in this paper that in very severe cases decapsulation of the kidneys may be a life-saving measure.

In view of Pons and Custer's findings, further observations on the histology of the brain should be undertaken.

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ABSTRACTS

In this section of the Journal some of the more important articles on subjects of interest to clinical pathologists are selected for abstract, and these are classified into four sections: bacteriology; biochemistry; haematology; and morbid anatomy and histology.)

BACTERIOLOGY

The Effect of Water Soluble Lipids on the Growth and Biological Properties of Tubercle Bacilli. DUBOS, R. J., DAVIS, B. D., MIDDLEBROOK, G., and PIERCE, C. (1946). *Amer. Rev. Tuberc.*, **54**, 204.

The slow rate of growth of tubercle bacilli in the conventional fluid culture media has been overcome by the use of a synthetic basal medium to which is added 0·05% "tween 80" and 0·1 to 20% filtered sterile serum albumin (conveniently 0·25% bovine serum fraction V, prepared as a powder). "Tween 80" is a polyoxethylene derivative of sorbitan mono-oleate, which is water-soluble and heat-stable. Inoculation of 5 ml. of this medium in 25-mm. test-tubes leads to rapid diffuse submerged growth in response to very small amounts of tubercle bacilli. It is believed that the "tween 80" acts as a wetting agent and that oleic acid is split off by a bacterial lipase to provide a readily available foodstuff; free oleic acid is, however, toxic, but this toxicity can be neutralized by the albumin. By adding 1·5% agar to this fluid medium and using 0·5% albumin, a solid medium is obtained that will give surface colonies from inocula in 1 to 2 weeks. Tubercle bacilli growing diffusely in the liquid medium retain their typical morphology and staining characteristics; they are viable for long periods and remain fully virulent. Such bacilli can be used for inoculation into experimental animals and into chick embryos, and they can produce, as well as react specifically with, immune sera. The medium can also be used for primary isolation of tubercle bacilli from pathological material.

[The use of water-soluble lipids is likely to revolutionize the techniques of culture of *Myco. tuberculosis.*]

P. D'Arcy Hart.

Stevens-Johnson Syndrome. Report of a Case. NELLEN, M. (1947). *Lancet*, **1**, 326.

A 20-year-old male was admitted to hospital with a 4 days' history of severe malaise, sore throat, productive cough, and vomiting; soreness of the eyes with a discharge had appeared 2 days before. On admission to hospital his temperature was 103° F., pulse rate 100, and respiration rate 30. He had (1) bilateral conjunctivitis with vesicles on the conjunctiva and a mucopurulent discharge; (2) ulceration of the nares and pustules on the upper lip; (3) discrete adherent white patches up to ¼ inch in diameter on the inner sides of both cheeks and on the tonsils; and (4) numerous rhonchi over both lungs. A throat swab showed "no haemolytic streptococci, no *Corynebacterium diphtheriae*, no Vincent's organisms"; the blood count was normal and the Wassermann and Kahn reactions negative. Two days later he developed (5) a purulent discharge and (6) vesicles (containing a pale sterile fluid free from cells) on an erythematous base on the dorsum of the arms; after 2 days the vesicles had spread to the lower limbs and trunk. They varied in size and rapidly developed dark crusts. The temperature ranged from 102° to 104° F. and the fever did not respond to sulphamezathine, so 6 days after admission penicillin, 15,000 units intramuscularly every 3 hours, was given and the condition improved. The corneae remained clear; irrigations with boric acid lotion and eye drops of 10% "albucid" (sulphacetamide) every 2 hours were prescribed. A fortnight after admission the patient developed for 2 days slight consolidation at the right base with a pleuritic rub, cough, and viscid sputum. A throat swab at this time grew *Staphylococcus aureus*; biopsy of a vesicular lesion showed "slight vascular engorgement of the corium and a little oedema of the Malpighian cells"; blood culture was sterile. Three weeks after admission the eyes were clear, the skin lesions were only brown discoloured patches, and 10 days later the mouth was clear and the urethral discharge had ceased, but the prepuce was so densely adherent to the glans penis that circumcision was performed.

Henry Cohen.

A Report of an Epidemic of Mild Lymphocytic Meningitis in Burma. KIBBE, F. W., and BREADENKOPF, W. G. (1946). *Johns Hopk. Hosp. Bull.*, **79**, 365.

Twenty-three cases of mild lymphocytic meningitis are reported; they probably formed about half those occurring in an epidemic among units served by a U.S. general hospital in Burma. The symptoms were: severe bilateral bursting frontal headache; "eye-ache" and pain on moving the eyes, fatigue, weakness, and nausea; vomiting; inconstant prodromal symptoms; fever (100° to 103° F.); sweating; conjunctival congestion; tremor; and neck rigidity, but not so marked as in bacterial meningitis. Reflexes, ocular muscles sensation, and mentality were normal, and there were no convulsions. Eight cases showed moderate lymphocytosis, the greatest shift being from 12% to 44%. There was no eosinophilia. Four patients while in hospital showed a probably significant heterophil antibody agglutination, and of 20 readmitted cases, seen 2 months after the onset, 13 gave agglutination titres of 1 in 7 to 1 in 224. Complement-fixation and virus neutralization tests for

lymphocytic choriomeningitis, mumps, dengue, and three types of encephalitis were negative. The cerebrospinal fluid was abnormal in all cases. Cell counts varied from 0 to 490 per c.mm., lymphocytes predominating except in the case with the highest count. Protein varied from 29 to 70 mg. per 100 ml. There was no relation between these findings and the severity of the symptoms, and in 4 cases the fluid was normal 4 weeks later. There was no evidence of spinal block, though manometric pressure determination could not be made.

The infection is regarded as being due to a virus, in the absence of bacterial, spirochaetal, or chemical factors, or of an associated disease likely to produce a reactive meningitis. The frequency of the heterophil reaction suggests infectious mononucleosis, some features of which were present. The test is, however, apparently non-specific, and a number of epidemic fevers in Japan give a positive Paul–Bunnell reaction. Poliomyelitis is unlikely, and tests for types of encephalitis were negative. The dengue group of diseases also appeared unlikely to be implicated. The absence of neurological dysfunction makes lymphocytic choriomeningitis again improbable. The disease is accordingly classed among the group of benign meningitides named "acute aseptic meningitis," in only one-third of which have specific viruses been found. If any virus were isolated or identified it would probably fall into the dengue group. *W. A. Bourne.*

An Outbreak of Trichinosis in New York City with Special Reference to the Intradermal and Precipitin Tests. SHOOKHOFF, H. B., BIRNKRANT, W. B., and GREENBERG, M. (1946). *Amer. J. publ. Hlth.*, **36**, 1403.

The consumption of pork products from a single wholesaler was held responsible for an outbreak of trichiniasis, involving 84 cases, occurring in New York in the early part of 1945. The values of the precipitin and intradermal tests as aids to diagnosis during the actual illness are considered. Fifty out of the 84 of the sufferers had eaten a sausage preparation called mettwurst. None of the pork products consumed had been subjected to the usual process of freezing for 20 days at 5° F.

The incubation period varied between 2 and 35 days, but was usually between 5 and 17 days. Attention is drawn to the fact that in this, as in most of the large-scale outbreaks of this disease in recent years, there were no fatalities. The main symptoms were oedema of the eyelids, muscle pains, and fever. Gastro-enteritis, generally considered an early condition, was present in only 7 cases. Differential blood counts were carried out in 72 cases; in 64 eosinophils were found to exceed 10% of the total white cells, and in only 1 of the remaining 8 was the eosinophil count less than 5%. The eosinophil count is considered the most useful single laboratory test in the early diagnosis of trichiniasis, and if eosinophilia is not present the diagnosis should be doubted unless the infecting organisms are isolated.

The precipitin reaction was performed in 40 cases and gave a positive result in 33, with 5 doubtful positive reactions. The intradermal reaction with 1 in 10,000 dilution of trichinella antigen was performed in 21 of the 84 cases and gave a positive result in 15 in some after repeated tests. The earliest positive reaction was obtained on the sixth day of illness, but the best results were achieved after the end of the second week, which makes the test somewhat limited in its use. The authors question the generally accepted view that the skin test becomes positive before the precipitin test, as their results indicate the reverse. The advisability of using both tests in all doubtful cases is suggested. Muscle biopsies were not carried out because it was considered that the other available tests gave positive reactions earlier in the illness. *H. C. Maurice Williams.*

False Positive Kahn Reactions. Loss of Titer on Storage of Serum in Ice Box. LUBITZ, J. M. (1946). *Amer. J. clin. Path.*, **16**, 768.

It had been noted that sera from patients after smallpox vaccination which gave false positive Kahn reactions tended to become negative when exposed to ordinary room temperatures for several days. The author tested the sera of 59 syphilitic patients (92 sera and 219 tests) over a period of 2 to 6 months with the quantitative Kahn test; the sera were stored at +2° to +4° C. Of the 92 sera, 25 (27·1%) showed a moderate loss of titre, 10 (10·9%) showed a gain, and 57 (62·0%) maintained a constant titre. He also tested two groups of false positive sera. Group I consisted of 13 patients with smallpox, 15 with malaria, 5 with upper respiratory infections, 2 with scarlet fever, and 1 each with tuberculosis of bone, infectious mononucleosis, gonococcal arthritis, lymphogranuloma venereum, and a "general biologic reaction"; 109 sera were taken and 208 tests carried out; all showed a decrease of titre or became negative at various periods, usually within a month. Group II consisted of 1 patient with pinta whose serum maintained its titre for 3 months, and 5 with leprosy; 2 sera maintained and 4 lost titre. Illustrative cases with results of serum tests are given in seven tables. That the serum from the patient with pinta maintained its titre is not considered surprising, since the disease is due to a spirochaete; the reactions of the leper sera are not so easily explained. The loss of titre in general may be due to non-specific antibody being more labile, or to an inhibitor, present in the serum, becoming activated: the difference in behaviour of syphilitic and non-syphilitic sera suggests that the two types of antibody are not the same. *T. E. Osmond.*

Comparison of the *in Vitro* Antigonococcal Actions of Penicillins G, F, K, and X. NELL, E. E., and HILL, J. H. (1947). *Amer. J. Syph.*, **31**, 14.

In this comparison the *in vitro* activity of penicillin G and X (both pure crystalline) and of F and K (approximately 90% pure) has been tested against freshly isolated cultures of gonococci obtained from a large number of patients. The authors are doubtful whether the gonococcus is an appropriate organism for standard testing and consider that the meningococcus might be more suitable. The tests indicate that pure crystalline penicillin is 7·3 times more efficient *in vitro* than is pure crystalline G against freshly isolated gonococci from 76 different patients, and that 90% pure penicillin is 3·3 times as efficient against the same organisms. Pure crystalline G and 90% pure K were also tested against gonococci from 40 different sources, and it was shown that the average *in vitro* efficacy of K was 1·5 times that of G.

[It will be appreciated that the fate of K in the body would discount its use as a practical therapeutic agent. The authors rightly urge that further clinical trials of crystalline penicillin X in gonorrhoea should be made as soon as possible. Their technique is ingenious and the paper should be read in the original by those who are interested.] *G. L. M. McElligott.*

BIOCHEMISTRY

Absorption and Excretion of Water. The Antidiuretic Hormone. VERNEY, E. B. (1946). *Lancet*, **2**, 739.

In the dog the peak of the diuresis which follows the ingestion of 250 ml. of water occurs after 50 minutes, but the absorption curve reaches the 250-ml. level at 35½ minutes. Subtraction of the water-excretion curve from the absorption curve gives the water load curve, which represents the amount of water temporarily held in the tissues in excess of the optimal amount. The peak of the water-load curve occurs 15 minutes earlier than that of the excretion curve. There is therefore a delay of 15 minutes between the maximal stimulus to excretion and the maximal response of the kidney to the stimulus. The diuresis is inhibited by muscular exercise, emotional stress, or a rise in the osmotic pressure of the carotid arterial plasma.

The inhibitory effect of exercise is accompanied by an increase in the chloride, nitrogen, and pigment content of the urine. This inhibitory effect is only apparent and is really due to emotional stress, as emotional stress produced by mild faradism without exercise gives rise to a similar inhibition, which might be due to vasomotor changes, though the rise in chloride excretion is against such an interpretation. This hypothesis is disproved by the fact that the inhibitory response is unaltered by complete denervation of the kidneys. The argument that the inhibition may be due to release of adrenaline is disproved by the fact that the response is not altered by the removal of one adrenal gland and the denervation of the other. Moreover, occlusion of the renal artery, whether complete or partial, causes a different response, the inhibition of urinary flow rising suddenly as soon as the compression ceases. Lastly, direct measurement of renal blood flow during emotional stress demonstrates the independence of the response.

Comparison of the inhibitory response to emotional stress with that due to the intravenous injection of adrenaline and of posterior pituitary extracts shows significant differences. Adrenaline produces a sudden fall in urinary excretion and an almost equally sudden recovery, a response unlike that of emotional stress. On the other hand, a suitable dose of posterior pituitary extract produces a response identical with that of emotional stress. There is a latent period (absent in experiments with adrenaline) followed not only by a decrease in urinary excretion but by an increase in urinary chloride and nitrogen. The return to normal is more gradual than after adrenaline injection. Exact similarity between the responses to emotional stress and to injection of posterior pituitary extract does not prove a causal identity. The proof is found in the fact that the polyuria which follows removal of the posterior pituitary subsides after a week. Thereafter emotional stress fails to elicit the typical response, which, however, occurs as usual after the injection of posterior pituitary extract.

Increased activity of the sympathetic nervous system may overcome the inhibitory effect of the posterior pituitary. The action of adrenaline is not specific, tyramine being as effective. It is probable that increase of cerebral blood flow is the common factor preventing release of the antidiuretic substances. It seems, therefore, that low sympathetic tone associated with noxious stimuli is favourable to the prolonged secretion of antidiuretic substance. The release of this substance is caused by such mild disturbances of the central nervous system that it becomes important to determine whether the secretion is varying continuously under the control of some factor in the animal's internal environment the maintenance of which within a narrow physiological range is important for the animal's welfare. It seemed probable that this factor is the osmotic pressure of the carotid plasma, as shown by the finding that the injection of hypertonic solutions into the carotid artery causes an inhibition of urinary flow, the magnitude of the response varying with the tonicity of the solution at constant rate and period of injection and with the period of injection at constant volume and tonicity. This effect disappears after removal of the posterior pituitary but can be mimicked by the injections of posterior pituitary extract. It can be produced by the injection either of sodium chloride or of iso-osmotic dextrose, and is therefore dependent on the osmotic pressure and not specifically the substance. Comparison with the effects of posterior pituitary injections demonstrates that increase in blood chloride of 8 mg. per 100 ml. causes the pituitary to secrete 1 μU. per second. There can be little doubt that the secretion of the posterior pituitary is a hormone in the true sense, its liberation being continuously governed by the contemporary concentration of chloride and possibly of other osmotically active substances in the arterial plasma. The control is presumably effected by osmoreceptors connected by nervous paths with the pituitary.

[This important paper should be read in full.]

Raymond Greene.

Biochemistry of Inflammation. MENKIN, V. (1947). *Lancet*, **1**, 660.

The author believes that the clinical features of acute inflammation are due to the liberation of "biochemical substances" from injured cells. In this paper he summarizes his extensive work on this subject and describes the five substances he has so far isolated from inflammatory exudates.

Leukotaxine increases capillary permeability, so that plasma proteins, dyes, colloids, and bacteria readily escape into and are concentrated in the injured area. The author has isolated this in a relatively pure form; it seems chemically not to be a histamine-like substance, as postulated by Lewis, but a relatively simple polypeptide. It is positively chemotactic to polymorphonuclears, but does not induce general leucocytosis. Leukotaxine is abundant in the rabbit's succus entericus, and may be found in such fluids as the contents of ovarian cysts.

L.P.F., a leucocytosis-promoting factor contained in inflammatory exudates, causes leucocytosis and hyperplasia of marrow granulocytes and megakaryocytes. The author has also isolated this substance, which appears to be a pseudoglobulin and not present in normal serum.

Necrosin is associated with the euglobulin fraction of inflammatory exudates but not of normal serum. Intradermal injection of necrosin causes an intense inflammatory reaction followed by superficial necrosis; the lymphatics are occluded by fibrin, and blood vessels may be thrombosed. Intravenous injection may cause focal necrosis of the liver, pleural effusion, intestinal haemorrhages, or renal changes. Necrosin is lethal to mice.

Pyrexin is another factor in the euglobulin fraction of inflammatory exudates which induces fever when injected intravenously into mice. Pure necrosin is not pyrogenic. Injection of the whole euglobulin fraction of inflammatory exudates causes leucopenia as well as necrosis and pyrexia. Vomiting, diarrhoea, and "general apathy" may also occur. The leucopenia seems to be

due to "a trapping of the leucocytes in lungs, liver, and spleen." It is ascribed to a specific *leucopenic factor*.

[This paper gives little idea of the stimulating qualities of Menkin's book, *The Dynamics of Inflammation*. The author's postulate, that the effects he describes in this paper are due to definite chemical entities constantly present as such in inflammatory exudates, may not gain general acceptance until much confirmatory evidence is available.] *Martin Hynes.*

Urinary Excretion of Phosphatases in Man. BURGEN, A. S. V. (1947). *Lancet*, **1**, 329.

Urinary phosphatase was determined by the hydrolysis of monophenyl phosphate and the estimation of liberated phenol by the diazo method of Theis and Benedict, which is unaffected by uric acid. The daily excretion of phosphatases in the urine of 50 males and 25 females without genito-urinary disease was estimated. Alkaline-phosphatase excretion was low and irregular and showed no significant variation with age or sex. The average daily acid-phosphatase excretion in females is about 50 units, irrespective of age. The male acid-phosphatase excretion level is similar in childhood, but rises sharply at puberty above that of females, reaching a maximum of 350 units between 30 and 40 years and returning to the prepubertial level in old age. The normal rate of haemolysis will explain female urinary excretion of acid phosphatase, but the acid-phosphatase excretion increase in the male during the reproductive period is usually assumed to come from the prostate. Samples collected over 24 hours from 3 men by suprapubic cystotomy suggest, however, that part of the excess acid phosphatase is excreted through the kidney. No alteration in phosphatase excretion was found in disease of the prostate or in nephritis. *E. F. McCarthy.*

The Nicotinamide Saturation Test. ELLINGER, P., and HARDWICK, S. W. (1947). *Brit. med. J.*, **1**, 672.

Urine samples collected every 24 hours for 11 days from 1 diabetic and 5 healthy persons were examined for nicotinamide methochloride by Coulson, Ellinger, and Holden's method (*Biochem. J.*, 1944, **38**, 150). The subjects were given oral doses of 100 mg. of nicotinamide after the third and ninth urine collection, and of 1 g. of methionine every 12 hours on the seventh to eleventh day. The methionine did not affect the methochloride excretion rate, but when the dosage of nicotinamide was prolonged so as to deplete the body stores of "methyl-donators," added methionine increased the urinary response. The effect was demonstrated by experiments on groups of 5, 8 (3 pellagrins), and 8 (3 pellagrins) mental patients, whose 24-hour urine samples were collected for 14 days. The patients received either oral or subcutaneous doses of nicotinamide after the third to the thirteenth urine collection and oral doses of 1 g. of methionine every 12 hours on the ninth, tenth, and eleventh days of the test. The effect of methionine was greater after a daily intake of 500 mg. of nicotinamide than after one of 100 mg.

The saturation test described by Ellinger, Benesch, and Hardwick (*Lancet*, 1945, **2**, 197) would be affected by a depletion of the body reserves of "methyl-donators," and the following modified test is recommended. Twenty-four-hour urine samples are collected for 18 days and the urinary nicotinamide methochloride is estimated. Doses of nicotinamide (100 mg.) are administered subcutaneously after the third and twelfth, orally after the sixth, and rectally after the ninth urine collection, and the percentage of ingested nicotinamide excreted during each 24- and 72-hour period after dosing is calculated. The results obtained for 6 physically fit and 3 pellagrin mental patients are discussed. The urinary response to nicotinamide administered rectally was slightly less than to that given orally, showing that nicotinamide can be absorbed from the lower intestine. *J. E. Page.*

Hydrogen Ion Concentration (*p*H) of Normal Vaginas. KARNAKY, K. J. (1947). *West. J. Surg. Obstet. Gynec.*, **55**, 103.

The author used the Beckman *p*H meter and Rakoff's vaginal electrodes to study vaginal *p*H in 52 pregnant and 15 non-pregnant women. This report differs from previous ones in that the author has determined the *p*H in different sites of the vagina—namely, anterior fornix, posterior fornix, and left and right lateral walls, the averages for these being 4.26, 4.37, 4.34, and 4.46 respectively. In non-pregnant women the average was 4.34, while in pregnancy the figure was 4.38. The over-all *p*H average in apparently normal vaginas varies between 3.27 and 4.99. *C. W. Kimbell.*

Use of a Tourniquet to Prolong the Effect of Penicillin. CARLINFANTI, E., and MORRA, F. (1947). *Lancet*, **1**, 521.

The rate of absorption of penicillin injected subcutaneously into thigh or arm can be considerably slowed by exerting light pressure with a rubber tourniquet proximal to the site of injection. Results comparable to those obtained with oil-beeswax preparations can thus be achieved. The penicillin concentrations in the blood were estimated by the capillary-tube method of Fleming and by another method devised in St. Mary's Hospital, London, with phenol-red as an indicator of the growth of staphylococci. Trials showed that the tourniquet should be applied for times varying from 1 hour after injections of 10,000 units to 5 hours after 100,000 units. With this method of administration the number of injections can be reduced to three of 75,000 units in 24 hours or to five of 100,000 units in 48 hours without letting the penicillin level fall below the lowest effective one. *Geoffrey McComas.*

Estimation of Penicillin in Serum. Use of Glucose, Phenol Red, and Serum Water. FLEMING, A., and SMITH, C. (1947). *Lancet*, **1**, 401.

A micro-method is described for the estimation of penicillin in serum; it is a modification of previous methods described by Fleming and by Dr. J. Fielding. The medium used consists of serum (human, horse, sheep, or ox) 2 ml., 10% glucose solution 2 ml., water 6 ml., and phenol red, saturated solution, 0.25 ml. This can be sterilized either by steaming in bulk or by boiling for a few minutes. A suitable quantity of the medium is inoculated with the test organism (5 ml. of a 24-hour broth culture to 1 ml. medium); the authors use a haemolytic streptococcus, which produces acid more quickly than staphylococcus. Serial dilutions of the serum to be tested (25-ml. volumes) are made in the inoculated medium on the surface of a paraffined slide. Glass capillaries made from soda glass, not from hard

glass, are taken, and each of the drops is touched with the end of a capillary tube so that the fluid runs into it. The tube is sealed (this can be omitted) and placed horizontally on a "plasticine"-covered slide and incubated. Tubes in which the streptococci have grown are bright yellow and show a heavy precipitate; tubes in which growth has been prevented by penicillin are red or red-violet. The test can also be done as a macro-method in small test-tubes. The authors have found this method rapid and convenient; they discuss the reasons for adopting this procedure and the permissible modifications.

[Presumably the number of units of penicillin present is read by comparison with a standard set of tubes containing known concentrations of penicillin.]

F. Hawking.

17-Ketosteroids in the Diagnosis of Adrenal Tumors. JOHNSON, H. T., and NESBIT, R. M. (1947). *Surgery*, **21**, 184.

Urinary 17-ketosteroids can be divided into *alpha* and *beta* fractions, the *alpha* produced by the adrenal cortex and testes, the *beta* by adrenal cortex only. The authors report 3 cases of adrenal cortical carcinoma with markedly elevated total 17-ketosteroids in which differentiation into *alpha* and *beta* fractions was of value. The first case was in a woman of 31 years with menstrual disturbances, gain in weight, hypertrichosis, and a mass in the right upper quadrant of the abdomen. Total urinary 17-ketosteroids amounted to 75 mg. per day (*alpha* 19.7, *beta* 55.3). (Normal for adult women: total 2.7 to 8.1; 5 to 15% *beta*.) A carcinoma of the right suprarenal was removed. Ten days after operation total 17-ketosteroids measured 3.8 mg. per day. The patient was well one year after operation. The second case was in a 2-year-old girl with pubic hair and a large clitoris. Excretory urograms showed a right adrenal tumour depressing the right kidney. Urinary 17-ketosteroids measured 78.5 mg. per day (*alpha* 15.7, *beta* 62.3). (Normal for a child aged 4 to 7 years: average of 1.3 mg. per day.) An adrenal cortical adenoma undergoing carcinomatous change was removed from the right side. Ten days after operation 17-ketosteroids measured 0.9 mg. per day. The child was well 6 months after operation, but there was only slight retrogression of pubic hair. The third case was in a man of 43 with an inoperable carcinomatous mass in the left adrenal region and no hormonal symptoms; 17-ketosteroids amounted to 86 mg. per day (*alpha* 36.6, *beta* 49.4). (Normal for adult man: 3.4 to 15 mg. per day.) The amount fell with radiotherapy, which produced clinical improvement, but rose again with re-growth of the tumour. The patient was discharged as unfit for further treatment.

D. H. Patey.

Mechanism of Hydrogen Peroxide Formation by Spermatozoa and the Role of Amino-acids in Sperm Motility. TOSIC, J. (1947). *Nature, Lond.*, **159**, 544.

Bull spermatozoa lose respiratory activity and motility when kept under aerobic conditions in egg-yolk medium. This inhibition is due to hydrogen peroxide, which is formed by enzymic action for some dialysable constituent of the egg yolk. The chemical behaviour of this constituent during attempts at its isolation suggested that it is an amino-acid. *l*-Tryptophane, *l*-tyrosine, and *l*-phenylalanine can replace egg-yolk medium and in the presence of living spermatozoa give rise to hydrogen peroxide. Only these three amino-acids of the many tested had this property. Such specificity of an *l*-amino-acid-oxidase is unusual. The adverse effects of hydrogen peroxide on sperm respiration and motility are produced with concentrations that are chemically undetectable, but appreciable since the effect is abolished by the addition of catalase.

[The finding will be useful in arranging optimal conditions for the storage and transport of sperm for artificial insemination, but probably has no physiological importance. Judgment on this will await more detailed description of the aerobic conditions needed for the enzymic activity.]

P. C. Williams.

Determination of Sex Hormones by the *m*-Dinitrobenzol Reaction. (Bestimmung von Keimdrüsenhormonen mittels der *m*-Dinitrobenzolreaktion.) ZIMMERMAN, W. (1946). *Schweiz. med. Wschr.*, **76**, 805.

This is a review of the dinitrobenzene assay of sex hormones and its applications in physiological, biochemical, bacteriological, and clinical research. The author's method for the assay of sex hormones in urine and blood is given in detail. The method has also been worked out for the assay of androgenic hormones in blood.

M. B. Klein.

HAEMATOLOGY

Genetic and Constitutional Causes of Foetal and Neonatal Morbidity. LEVINE, P. (1946). *Ann. N.Y. Acad. Sci.*, **46**, 939.

In some 92% of cases of erythroblastosis foetalis the mother is Rh-negative—that is, all her cells are not agglutinated by the anti-Rh_0 (anti-D) serum and her serum contains anti-D agglutinins or blocking antibodies. In about 8% of cases of erythroblastosis foetalis the mother is Rh-positive; in these cases the factor responsible is one of the other Rh antigens, such as Hr[c] or in some cases the ordinary A and B antigens or possibly other rare blood group factors. When A or B are responsible " tests of the affected infant's saliva or the deceased infant's organs should reveal the infant to belong to the non-secretor type." "The demonstration of specific increase in anti-A or anti-B agglutinins is contributory evidence." [These two statements are difficult to reconcile, since it has been shown by Smith that iso-immune responses to A and B during pregnancy only occur when the infant is a "secretor."] The statistical proof of the part played by A and B in causing occasional cases of erythroblastosis foetalis is given in the following table:

Matings	Compatible %	Incompatible %
Random	65	35
215 Rh-negative mothers*	75	25
28 Rh-positive mothers*	50	50

*Mothers of erythroblastotic infants.

In this table incompatible matings are defined as those in which the father's blood contains A or B and the mother's serum contains corresponding anti-A or

anti-B agglutinins. The higher value for incompatible matings in the Rh-positive mothers indicates that iso-immunization by the A or B factors can explain at least some of the exceptional cases. That incompatible matings of this kind also sometimes cause early foetal death is suggested by the following figures:

Matings	Compatible	Incompatible
115 with 2 or more miscarriages	46%	54%
43 with 2 miscarriages or stillbirths	44%	56%

"These figures are highly suggestive and probably significant." Obviously the statistical proof cannot be expected to be so convincing as in the case of the connexion between the Rh factor and erythroblastosis foetalis, since there are certainly many causes of abortion and stillbirth besides iso-immunization to A and B. Further support for the view that immunization against A and B occasionally causes foetal death is provided by the observation that the proportion of A children in matings between A fathers and O mothers is slightly smaller than that produced by matings between O fathers and A mothers. Erythroblastosis foetalis occurs very rarely in the first-born; however, transfusion of Rh-negative women at any time before pregnancy tends to increase this occurrence, especially in its more severe form.

Erythroblastosis Foetalis in the First-born of Rh-negative Women

	Transfusion History	
	Previously Transfused	Never Transfused
Severity of disease:		
Mild	1	4
Severe	5	4
Foetal deaths	10	1
Total	16	9

All persons giving transfusions of blood should be aware of this most serious risk.

[This paper is a summary of information previously published, but it is worth reading even by those who are already familiar with Levine's contributions. Only a few points in it can be referred to in this abstract.]

P. L. Mollison.

Isolation and Purification of Blood Group A and B Substances; Their Use in Conditioning Universal Donor Blood, in Neutralizing Anti-Rh Sera, and in the Production of Potent Grouping Sera. WITEBSKY, E. (1946). *Ann. N.Y. Acad. Sci.*, **46**, 887.

At present two different preparations of blood-group-specific substances are available commercially in the U.S.A.: (1) the A specific substance isolated from hog stomach, and (2) the AB specific substance isolated from horse stomach. Both are chemically free from protein, and neither will sensitize guinea-pigs. These purified preparations are of practical use:

1. In neutralizing the anti-A and anti-B iso-antibodies of blood of group O. There is now abundant evidence that anti-A and anti-B agglutinins in group O blood frequently cause some degree of destruction of the recipient's erythrocytes when the latter are of group A or B; occasionally the degree of destruction is sufficient to cause severe or even fatal reactions, a danger that can be avoided by the addition to the blood of small amounts of AB substance to reduce the strength of the antibodies to a harmless level.

2. In suppressing anti-A and anti-B iso-agglutinins in anti-Rh sera. In making Rh tests, sera obtained from human beings who have been sensitized by pregnancy or transfusion are most frequently used. These of course often contain anti-A or anti-B agglutinins, which must be removed or neutralized before the sera can be used to test AB cells. Complete suppression of the anti-A and anti-B agglutinins can be effected by quite small amounts of the purified group-specific substance.

3. In production of potent grouping sera. As little as 0·1 mg. of blood-group-specific substance injected intravenously into human beings will often produce a tenfold to one-hundred-fold increase in the original iso-agglutinin titre. At the same time the avidity of the sera is increased (a minority show a small or negligible response).

P. L. Mollison.

The Clinical Significance of Rh Antibodies (Rh Agglutinins and Blocking Antibodies) in the Sera of Rh-negative Mothers; A Study of 179 Cases. HOWARD, J., LUCIA, S. P., HUNT, M. L., and MCIVOR, B. C. (1947). *Amer. J. Obstet. Gynec.*, **53**, 569.

In an effort to correlate the laboratory findings with the clinical condition of the babies at birth, the authors studied the antibody titre of 179 Rh-negative expectant mothers. The maternal sera were examined for complete and incomplete antibodies. The investigations were carried out in 1944–5, and the latter type of antibody was detected by its blocking activity with Rh_0 (cDe/cde or cDe/cDe) cells and not by the Race-Coombs test.

The 179 pregnancies are divided into four groups: group 1, normal Rh-negative children; group 2, normal Rh-positive children; group 3, "subclinical" haemolytic disease of the newborn; group 4, severe haemolytic disease of the newborn. In group 1 (61 cases), only 9 showed isolated complete anti-Rh antibodies, probably carried over from previous pregnancies. Group 2 (78 cases) showed no ante-partum antibody production, but a small titre of either complete or incomplete antibody might appear post partum, reaching a maximum usually in the second week. There was no significant difference between primiparous and multiparous women. Group 3 (17 cases) showed production of antibodies in the last 12 weeks of pregnancy. Ante partum, in this group, there appeared to be a predominance of blocking antibody, but the amount of this fell after delivery. Of the 17 mothers, 3 were primiparous and 14 multiparous —a significant difference. In group 4 (20 cases) antibodies were always demonstrated ante partum and usually the amounts of the two varieties (complete and incomplete) varied inversely; in no case did they rise or fall together. Most commonly the complete antibodies diminished as pregnancy advanced and the incomplete antibodies increased.

In 4 cases stillborn children were delivered, and in 3 of these complete antibodies were demonstrated in the maternal serum. On the other hand, in only 3 of 12 infants born alive were blocking antibodies not demonstrated in the mother's blood. The authors point out that this contradicts Wiener's view that the incomplete antibody is of more serious import, and discuss the possibility that the incomplete antibody acts as a protective substance for the foetal red cells. The antibody titres in group 4 rose to a maximum about the second week post partum and then declined. *A. J. Buller.*

The Transition Forms of Blood Groups. HIRSZFELD, L. (1947). *J. Immunol.*, **55**, 141.

A review of the author's theory of the antigenic structure of the A_1 A_2 BO blood groups is presented. By parallel titrations of red cells of group A_1 against anti-O (goat anti-Shiga bacillus) and anti-A sera, several different forms can be separated. Some appear to have more O substance and less A substance and some the reverse, the total antigenic substance being constant. The reaction of A blood with anti-O serum does not depend on the blood being heterozygous O, for in testing a large number of samples of blood, many of which must have been A_1A_1 (18% in Poland), none was found which was completely negative with the anti-O serum. Further, the blood of known A_1A_1, A_1A_2, A_1A_3, and A_2B persons was agglutinated by the anti-O serum. The author therefore considers that the cause of the agglutinability "must be searched for in the structure of the A and B genes as such."

The hypothesis is put forward that there is a range of increasingly complete mutations stretching from O through $A_3 \rightarrow A_4 \rightarrow A_5 \rightarrow A_2 \rightarrow A_m \rightarrow A_r \rightarrow A_j$ to A_c. A_3 is so weak in A that the cold agglutinin anti-A is present in the serum; A_4 is what is usually thought to be a group O with anti-A missing from its serum. The stages A_m, A_r, and A_j correspond to the classical A_1 gene, and the hypothetical A_c is completely A. The mutations appear to be changes of similar nature, but of increasing extent. A parallel series is proposed from O to B_c. People who possess anti-O are considered to be A_cA_c, A_cB_c, or B_cB_c. The rarity of persons of these groups explains the infrequency of anti-O.

[It is a pity that this important paper is not more detailed; the conceptions of gene and genotype are not clearly distinguished, and from reading this paper alone one would be left wondering whether the bloods classified as A_m, A_r, and A_j were known AO heterozygotes, for, if they were not, it seems that A_mA_m might have as much A and O substance as A_jO, and be indistinguishable from it. This aspect is considered in *Ann. Inst. Pasteur*, 1940, **65**, 251 and 386. The author suggests that the direction of mutation is away from O towards A_c, and that O may eventually disappear. Such progressive evolution is not in accord with current doctrine. It also conflicts with Ford's view that the blood groups represent a balanced polymorphism (*Genetics for Medical Students*, 1942, p. 19). The evolutionary conjectures are not, however, as the author points out, an essential part of his theory. The theory does not seem capable of explaining the presence of α_1 in the serum of A_2B persons when this α_1 is active at body temperature. At least three such cases have been reported by experienced workers.] *R. R. Race.*

Substitution Transfusion : A New Treatment for Severe Erythroblastosis Fetalis. WALLERSTEIN, H. *Amer. J. Dis. Child.*, **73**, 19.

A method is described of substitution transfusion given immediately after birth in the sub-icteric stage of haemolytic disease of the newborn to prevent subsequent damage to the liver and brain. Hepatic damage in cases of icterus gravis by excessive red-cell haemolysis and a reactive liver haemopoiesis has been recognized. If this is to be avoided the end-products of haemolysis must be removed or their formation prevented. This may be done by withdrawal of the infant's Rh-positive cells and their replacement by harmless Rh-negative cells. Withdrawal and replacement should be carried out simultaneously. The technique is as follows: (1) Isotonic saline or pooled plasma is infused into an arm vein. (2) Blood (50 to 60 ml.) is withdrawn by syringe from the longitudinal sinus. (3) Rh-negative blood of a compatible group replaces the plasma or saline infusion. (4) When an estimated substitution of 80% has been accomplished the needle is removed from the fontanelle. The estimate is based on the infant's weight, counting the total blood volume as 10%. (5) The infusion is continued until 75 to 100 ml. of Rh-negative blood has been given in excess of the amount recovered, 10 ml. of 10% calcium gluconate being injected into the vein to counteract the citrate in the blood or plasma. The procedure takes from $\frac{1}{2}$ to $1\frac{1}{2}$ hours. Alternatively blood may be withdrawn from a small branch of the radial artery at the wrist. It is difficult to obtain enough blood from superficial veins. [Withdrawal of blood from the longitudinal sinus is not without its dangers in a jaundiced baby.]

Details of cases in which the prognosis was considered grave are given. Seven patients recovered and 2 died, both of whom might have recovered if treatment could have been started in the first 24 hours of life. Emphasis on anaemia as the indication for treatment is an error; the protection of the liver is the primary indication. About 30% of cases of haemolytic disease show liver damage and 10% kernicterus; until these changes can be accurately forecast, substitution transfusion should be used in all cases. If cases are to be selected the following are the most helpful criteria: (1) multiparity with a history of erythroblastosis in previous pregnancies; (2) serological studies; (3) during delivery, the presence of icteric amniotic fluid; (4) an excessively large, pale placenta; (5) excess of nucleated red cells in the blood in the cord. With such indications preparation should be made in advance and substitution therapy instituted as soon as the baby is born.

A. G. Watkins.

The Treatment of Lymphoblastic Leukemia with Crude Myelokentric Acid. MILLER, F. R., HERBUT, P. A., and JONES, H. W. (1947). *Blood*, **2**, 15.

Miller, in association with other workers, has previously shown that myelokentric acid and lymphokentric acid are present in varying amounts in the urine of patients with acute and chronic leukaemia, Hodgkin's disease, and lymphosarcoma. He has suggested that these substances are of fundamental importance in the abnormal processes of blood-cell production in the leukaemias, since they may constitute a balance mechanism in normal blood-cell proliferation and maturation. Lymphokentric acid is thought to bring

about lymphoid proliferation without maturation, while myelokentric acid by inhibiting proliferation of lymphoid cells allows them to mature. Maturation of myeloid cells is brought about by the action of lymphokentric acid, which inhibits their proliferation. The present paper discusses the effect of treatment of 8 cases of lymphoblastic leukaemia with myelokentric acid recovered from the urine or faeces, since on Miller's hypothesis there is a deficiency of this material in this condition. The authors claim 13 partial remissions. [Since no patient lived longer than 8 months the significance of the results is doubtful.] *Janet Vaughan.*

Comparison of the Effects of Massive Blood Transfusions and of Liver Extract in Pernicious Anemia. DAVIDSON, C. S., MURPHY, J. C., WATSON, R. J., and CASTLE, W. B. (1946). *J. clin. Invest.*, **25**, 858.

Five patients with Addisonian pernicious anaemia were given rapidly repeated blood transfusions until the erythrocyte counts and haemoglobin values in three were within normal limits. The symptoms attributable to the diminished oxygen-carrying capacity of the blood were relieved, but not such complaints as anorexia, apathy, and other digestive disturbances; these disappeared with liver extract. This sequence is typical of the nutritional deficiency disorders and indicates that blood transfusion does not relieve the fundamental defect in pernicious anaemia.

Injection of liver extract into the patients with normal blood counts following transfusion failed to cause any reticulocytosis, thus showing that this phenomenon occurs only when the stimulus of anoxia is exerted on the bone marrow. After transfusion but before the administration of liver the "bone marrow megaloblasts characteristic of pernicious anaemia" disappeared. The authors believe that a "maturation arrest" due to nutritional deficiency exists in pernicious anaemia. That haemolysis may be a factor of importance in the causation of anaemia they do not, however, attempt to deny.

[From the context it seems probable that the authors mean by the word "megaloblast" the most primitive nucleated red blood cell and not a series of abnormal elements. It is in this latter sense that most haematologists now employ the term, and as it is customary to regard the bone marrow in pernicious anaemia as dysplastic, the "maturation arrest" hypothesis becomes untenable.] *R. Bodley Scott.*

Studies in Iron Transportation and Metabolism. V. Utilization of Intravenously Injected Radioactive Iron for Hemoglobin Synthesis, and an Evaluation of the Radioactive Iron Method for Studying Iron Absorption. DUBACH, R., MOORE, C. V., and MINNICH, V. (1946). *J. Lab. clin. Med.*, **31**, 1201.

The authors have studied by Hahn's technique the rate at which small doses of radioactive iron injected intravenously are used for haemoglobin synthesis. In healthy adult men utilization is almost complete 75 days after injection; normal dogs, however, used only 35 to 70% of the injected isotope. In iron-deficient patients and dogs there was complete utilization in 5 to 6 days. In hypoplastic anaemia less than 4% of the injected isotope was used; in pernicious anaemia in relapse utilization was insignificant until red-cell formation became active as a result of giving liver; it was then greatly accelerated and eventually became complete. In haemolytic anaemia the percentage of an injected dose of radioactive iron in the peripheral blood varied greatly from time to time.

The authors seek to explain some of these results on the hypothesis that iron recently stored, whether derived from absorption from the alimentary tract, from injection, or from breakdown of haemoglobin, is retained in a form mobilized more easily than iron which has been stored for longer periods. It is thus used selectively for current needs. They point out that as radioactive iron is not used completely for haemoglobin synthesis it is unsafe to use as a measure of absorption the proportion of an oral dose appearing in the blood, as has been done in the past. *R. Bodley Scott.*

Chemical, Clinical and Immunological Studies on the Products of Human Plasma Fractionation. XXXII and XXXIII. The Coagulation Defect in Hemophilia. An *in Vitro* and *in Vivo* Comparison of Normal and Hemophilic Whole Blood, Plasma and Derived Plasma Protein Fractions. LEWIS, J. H., DAVIDSON, C. S., MINOT, G. R., SOULIER, J. P., TAGNON, H. J., and TAYLOR, F. H. L. (1946). *J. clin. Invest.*, **25**, 870, 876.

The ability of normal plasma to accelerate the coagulation of haemophilic blood has long been recognized. This effect has been shown to be unrelated to any of the formed elements of the blood. This paper records the fractionation of haemophilic plasma by the Physical Chemistry Department of the Harvard Medical School and subsequent testing of the fractions for anti-haemophilic activity in comparison with those of normal plasma. Both plasmas were similar in detectable protein content, Tiselius diagrams, and fibrinogen and prothrombin content. Normal plasma and normal fractions I, II, III, and IV–1 contain a substance capable of accelerating clotting of haemophilic blood. This substance is absent from haemophilic plasma and its fractions. The fractions mentioned contain most of the globulins and the fibrinogen.

Further efforts (article XXXIII, p. 876) have been made to determine the protein fractions of normal plasma which have the power of accelerating the clotting of haemophilic blood. After removal of fibrinogen from Fraction I by heat, the residual solution retains undiminished its anti-haemophilic power.

R. Bodley Scott.

MORBID ANATOMY AND HISTOLOGY

The Evolution of Adenomas of the Large Intestine and their Relation to Carcinoma. HELWIG, E. B. (1947). *Surg. Gynec. Obstet.*, **84**, 36.

A careful study at 1,460 consecutive necropsies disclosed that in 139 (9.5%) adenomata were present in the colon or rectum. In 80 cases the tumour was single, in 59 multiple. There was a steady increase in the incidence after the age of 30, the incidence reaching a maximum in the eighth decade, when 25.8% of the males and 20.5% of the females were affected. The author found that he could not classify the tumours on their macroscopical or microscopical appearances. There was no recognizable difference from the adenomata of familial adenomatosis coli. Different cross-sections of the same tumour showed varying microscopical appearances. The tumours were prone to trauma and infection, inflammation, ulcers, small abscesses, and haemato-

mata, haemosiderin being found in them at times. Evidence of previous or present colitis was found in only one case in association with an adenoma, and so the author considers colitis an unlikely exciting agent and believes the tumours to be true neoplasms.

It was noted that adenoma was commonest in the sigmoid colon, as was carcinoma. Early carcinomatous change was found in 10 of the 139 adenomata. This might occur in any part of the tumour—stalk, base, or periphery. In one case 2, and in another 3, adenomata showed early carcinomatous degeneration. Three of the 10 also had manifest carcinoma associated with the malignant adenoma. In the 1,460 necropsies 20 manifest or obvious carcinomata of the colon were found, and 13 of these had either benign adenoma or adenoma with malignant transition associated with them. Two cases in which a very early carcinoma appeared to be arising from the mucous membrane were also seen, both of which were unassociated with either adenoma or manifest carcinoma elsewhere in the colon. Thus a significant association between adenoma and carcinoma is shown, and the conclusion is drawn that, while some carcinomata arise directly from the mucous membrane, the majority arise in adenomata.

[This valuable article would repay study in the original.] *N. Tanner.*

Some Experiences with Bone Tumours. BRAILSFORD, J. F. (1947). *Brit. J. Radiol.*, **20**, 129.

The author describes fully, with illustrations, a series of cases of bone lesions culled largely from his own observations. He describes a bone tumour in a young girl which, though radiologically diagnosed as a sarcoma, proved to be syphilitic. Here the onset 7 weeks after a blow at the site had suggested to the author the possibility of an infective lesion and caused him to advise against amputation. Another sarcoma-like tumour in the humerus of a child was accompanied by an apparently typical secondary deposit in the lungs. This precluded surgery, and sulphathiazole administration was advised. In 2 years, restitution of the bone to normal was complete and the "secondaries" had vanished. The author suggests that this may have been a manifestation of reticuloendotheliosis. A case of sarcoma in the femur of a young man showed seemingly good response to radiotherapy, but there was local recurrence within a year, and later, after amputation, skeletal metastasis. The author suggests that surgery, to be successful, should follow closely on radiotherapy. A further case of bone disintegration occurred at the lower end of the femur in a boy, aged 14, who was suffering from paralytic deformities. This showed gradual spontaneous consolidation and recovery. The lesion is considered, for reasons based upon the radiological appearance, to have been probably a neurotrophic joint condition allied to Charcot's disease.

That biopsy may prove misleading, and should not in the face of contrary radiological evidence be given too much weight, is the moral drawn from 2 further cases of sarcoma: biopsy material was reported to be inflammatory, but both cases developed secondary deposits. Two cases of osteoclastoma are described. The author states that radiotherapy may cure, but immobilization in plaster during the active stages is important. The first patient did well. The second was poorly immobilized, did not respond to treatment, and had eventually to undergo amputation for secondary haemorrhage. The patient is now otherwise well. Failure to give due weight to the radiograph by the surgeon in the case of a man of 58 with a swelling at the knee-joint resulted in amputation. The lesion proved to be a Baker's cyst. The radiograph showed typical osteo-arthritic changes only. The rapid onset of secondaries after diagnosis of a bone sarcoma in another case described suggests the hopelessness of surgical intervention, but against this is quoted Speed's case in which, after amputation of a sarcoma, secondaries in the lung became shrunken and hard in outline, and remained unchanged 10 years later.

The author concludes that clinical and radiological evidence must be carefully correlated, that biopsy may be misleading, and that too hasty recourse to surgery may be ill-advised. He makes a plea for more emphasis on clinical radiology and less upon physics during the training of students. *A. M. Rackow.*

Tumors of the Testis. A Report on 922 Cases. FRIEDMAN, N. B., and MOORE, R. A. (1946). *Mil. Surg.*, **99**, 573.

The authors report their study of 922 cases of testicular tumours collected at the Army Institute of Pathology (U.S.A.) between October, 1940, and May, 1946. Most of these occurred in men of military age, 18 to 38 years, although men up to 50 years of age were also included. The distribution of the tumours was: 35% seminomata, 35% teratocarcinomata (malignant teratoma), 19% embryonal carcinomata (including chorionic carcinomata), 4% adult teratomata, and 3% miscellaneous tumours. The incidence (corrected for army population in each age group) of teratocarcinoma was practically constant throughout the age groups, being approximately 1 per 100,000 men, whereas the incidence of seminoma rose with age. Structurally the most homogeneous tumours were the seminomata, although a small number of cases showed anaplasia and were difficult to differentiate from embryonal carcinoma. The authors believe that seminoma does not arise from spermatogonia but from primordial germ cells; they suggest that this tumour should be called "germinoma." It is emphasized that seminomata differ both structurally and biologically from embryonal carcinomata. The common varieties of the latter type of tumour are those which show glandular and papillary structures (adenocarcinoma and papillary adenocarcinoma), and those which resemble trophoblastic elements, usually cytotrophoblastic and occasionally syncytiotrophoblastic components of chorionic carcinomata.

Chorionic carcinoma (chorion-epithelioma) is regarded as a variety of embryonal carcinoma rather than a separate entity, because chorionic structures were observed also in predominantly embryonal carcinomata and because gynaecomastia and related endocrine changes were associated as often with embryonal carcinomata (or teratocarcinomata) as with chorionic carcinomata. Under the term teratocarcinoma the authors include all tumours which contain differentiated teratoid structures and foci of histologically malignant growth. Most of such tumours examined were mixtures of teratoma, embryonal carcinoma, and/or chorionic carcinoma. Teratocarcinoma is thought to result from the teratoid differentiation of embryonal carcinoma. Teratomata without histologically malignant elements should not be termed benign, because they often produce metastases. The metastases of seminomata consisted usually of the same type of cell as the primary growth, but occasionally the metastases showed the characteristics of embryonal carcinoma or teratocarcinoma.

The metastases of embryonal carcinomata were likewise similar to the primary growth, but in some cases the metastases had the structure of chorionic carcinoma, although there was no evidence of this in the primary growth. With teratocarcinomata 40% of the metastases consisted of embryonal or chorionic carcinoma and 60% of teratoid structures. The immediate prognosis is bad in embryonal and chorionic carcinomata and poor in teratocarcinomata and adult teratomata. In seminomata, in comparison with the other tumours of the testis, the immediate prognosis is good. *G. Popják.*

Microscopic Renal Calculi. ANDERSON, L. (1946). *Proc. Mayo Clin.*, **21**, 326.

Following up the work of Randall, who in 1941 described the finding of small macroscopic calcareous deposits on the surface of the renal pyramid in 20% of 1,100 pairs of kidneys, the author investigated microscopic calcareous deposits in the substance of the renal papilla. The diameter of these deposits is at least five or six times that of a normal tubular cell. He found them in all of 170 kidneys examined. Some of these organs were apparently healthy and some were diseased; all were removed from bodies more than 9 years of age at the time of death. Discussion of these findings concerns the possibility that these microscopic deposits may form the nidus for the formation of Randall's plaques, and so perhaps for renal calculi in certain circumstances. *Geoffrey Evans.*

The Cytology of Conjunctival Exudates. THYGESON, P. (1946). *Amer. J. Ophthal.*, **29**, 1499.

A study of the cytology of conjunctival scrapings, exudates, and follicular expressions may, in combination with the bacteriological report, be of great assistance in making a correct diagnosis in cases of conjunctival disease. The staining method recommended is by Giemsa, 1 drop of stock solution in 1 ml. of distilled water, applied for 1 hour after fixing in absolute methyl alcohol. Excess stain is removed by washing in two changes of 95% alcohol. A polymorphonuclear leucocyte reaction is found in most cases of acute and chronic conjunctivitis, the outstanding exception being diplobacillary infection. An eosinophil reaction is characteristic of all allergic inflammation and is found in hayfever conjunctivitis and in various drug cosmetic allergies as well as in spring catarrh. Mild eosinophilia is found in ocular pemphigus but not in phlyctenular conjunctivitis. There is also a basophil reaction in allergic inflammations of the conjunctiva, parallel with the eosinophilia, but this is not specific because it may also occur in such non-allergic inflammations as trachoma. Mononuclear cells, chiefly lymphocytes, are found in exudates from epidemic kerato-conjunctivitis and acute follicular conjunctivitis. There is a similar reaction to the typical virus infection, such as that due to herpes or molluscum contagiosum. Plasma cells are found in the exudates of trachoma. Keratinization of epithelial cells is found in vitamin A deficiency and also in kerato-conjunctivitis sicca. The author is unable to confirm previous observations that trachoma can be diagnosed from conjunctival cytology at an early stage, but states that the cytology of expressed trachoma follicles is characteristic. The predominant cell is the lymphoblast, which shows degenerative changes, and there are many macrophages (Leber cells). This typical appearance is due to the necrotizing action of trachoma toxins. *A. G. Cross.*

Anatomical and Histological Lesions of the Liver in Experimental Thyroxine Poisoning and in Hyperthyroidism. (Les lésions anatomiques et histologiques du foie dans l'intoxication thyroxinienne expérimentale et dans l'hyperthyroidisme.) GUYE, P., and RUTISHAUSER, E. (1947). *Ann. Anat. path. méd.-chir.*, **17**, 1.

This well-documented paper records experiments made on rabbits and post-mortem observations on 28 cases of human thyrotoxicosis. Histologically there is a basic similarity. In the severe cases there is generalized atrophy of the hepatic parenchyma with gross cytoplasmic deprivation (glycogenolysis); in the more extreme cases there is also necrosis, generally central and most obvious in the subcapsular zone. Intoxication of low intensity and short duration produces lesser parenchymatous lesions of reversible degree. If of longer duration, or repeated, the intoxication is followed by fibrosis, both primary (portal in situation) and secondary (in relation to the necrosis). *A. C. Lendrum.*

Histopathology of Polioencephalitis Hemorrhagica Superior (Wernicke's Disease). BAILEY, F. W. (1946). *Arch. Neurol. Psychiat., Chicago*, **56**, 609.

Five fatal cases of Wernicke's encephalopathy were studied histologically. All the subjects were said to have been chronic alcoholics, though there is no reference to the amount of alcohol, its nature (except in one case), or the duration of alcoholism. Naked-eye examination of the brain showed cortical atrophy in 4 of the cases and small haemorrhage in the brain-stem in 2. Microscopical examination revealed histological changes confined largely though not entirely to the periventricular grey matter of the midbrain and pons—throughout the hypothalamus, mammillary bodies, medial aspect of the thalamus, inferior colliculi, and to some extent in the floor of the fourth ventricle. In the affected areas the capillaries showed proliferation of endothelial cells and the perivascular spaces were enlarged. There were areas of old and fresh haemorrhages, perivascular and of different sizes. Focal areas of parenchymal necrosis varied from one to several millimetres in diameter. Within the areas of haemorrhage and necrosis, focal neuronal degeneration was present in all cases. In the affected areas astrocytes showed proliferation and degeneration, and to some extent transformation. Oligodendroglia was swollen, with multiplication of cells. It is considered that underlying the histological changes there is a deficiency of nutritional factors, the chief of which is thiamine. It is considered that thiamine lack interferes with carbohydrate metabolism, with resulting anoxia of the tissues.

[This is an interesting study. Although the biochemical explanation may be correct, no suggestions are offered as to why these changes have such a strictly circumscribed distribution.] *Hugh Garland.*